New insights into aerophagia, belching and gastro-oesophageal reflux

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New insights into aerophagia, belching and gastro-oesophageal reflux

Nieuwe inzichten in aerofagie, eructatie en gastro-oesofageale reflux

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. J.C. Stoof, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op donderdag 15 oktober 2009 des middags te 4.15 uur

door

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geboren op 9 juni 1981 te Almelo

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General introduction

1

GASTRO-OESOPHAGEAL REFLUX

Definition and prevalence

The backward flow of gastric contents into the oesophagus (gastro-oesophageal reflux) is a physiological phenomenon and occurs in every healthy subject approximately 30 to 60 times every day [1, 2].

Fortunately, these reflux episodes are usually not perceived nor do they cause damage to the oesophageal mucosa. Some patients however do sense reflux episodes, as heartburn, regurgitation or chest pain [3-6]. These symptoms occur very frequently in the Western society, as 15-20% of the general population suffers from heartburn and/or regurgitation at least twice a week [7-10].

In 2006, a consensus meeting in Montreal resulted in the most recent definition of gastro-oesophageal reflux disease (GORD), where it is defined as "a condition which develops when the reflux of stomach contents causes troublesome symptoms and/or complications" [5]. It is known that reflux symptoms can decrease the quality of life significantly [11-13].

Besides symptoms, gastro-oesophageal reflux can also induce mucosal damage. Reflux oesophagitis, peptic stricture, and intestinal metaplasia (Barrett's oesophagus) can all be provoked by the recurrent backflow of gastric contents into the lower oesophagus. Subsequently, these mucosal lesions may lead to other symptoms or complications, such as dysphagia and upper gastrointestinal bleeding or even to the development of oesophageal adenocarcinoma.

Pathophysiology of GORD

The pathophysiology of GORD is complex and only partially understood. Multiple factors may play a role in the development of reflux symptoms and injury of the oesophageal mucosa.

Gastro-oesophageal reflux is normally prevented by the so-called anti-reflux barrier. This barrier is a high-pressure zone that comprises two components: the lower oesophageal sphincter (LOS) and the crural diaphragm [14]. The LOS is a specialized segment of circular muscle in the oesophageal wall, located just above the gastro-oesophageal junction. This sphincter relaxes during swallows, allowing food and beverages to pass through into the stomach.

In addition to swallow-induced relaxations, the LOS exhibits a second type of relaxation which serves to vent intragastric air. This is necessary as with the consumption of food or beverages, a small amount of air is ingested during each swallow [15]. This ingested air accumulates and causes distention of the proximal stomach. To prevent the stomach from injurious dilatation, a reflex through the nervus vagus is mediated, resulting in a so-called transient relaxation of the lower oesophageal sphincter (TLOSR) [16-18]. During TLOSRs, gastric contents and air can

escape from the stomach into the oesophagus. The expulsion of air from the stomach to the outside world is known as belching or eructation.

The crural diaphragm is the second component of the anti-reflux barrier. Especially during inspiration, the contraction of the diaphragm contributes to the high-pressure zone, preventing gastro-oesophageal reflux to occur.

Normally, these two components are located at the same level and act together to establish the high-pressure zone. When these two components are physically separated, as the result of formation of a sliding hiatal hernia, the anti-reflux barrier is compromised. It is known that the distance between the LOS and the diaphragm is not constant. In patients with a small hiatus hernia intermittent complete reduction of the hiatus hernia occurs frequently [19]. A hiatal hernia is present in approximately 40-70% of patients with reflux symptoms, but also in 37% of asymptomatic subjects, indicating that the presence of a hiatal hernia does not equate to the presence of reflux symptoms [9, 20].

Patients with reflux symptoms generally have more gastro-oesophageal reflux than healthy volunteers, but a large overlap between these groups exists, indicating that the severity of the reflux itself does not explain the development of GORD [21, 22]. Besides the quantity, the characteristics of reflux episodes may also play a role, as reflux episodes are more often symptomatic when they reach the proximal oesophagus and when they are accompanied by gas [4, 23-25].

Another subject of interest is oesophageal hypersensitivity. The oesophagus can be sensitized to reflux by recurrent oesophageal exposure to acid or mechanical distension or by more remote stimuli such as duodenal lipid infusion [26-30]. Central sensitization also plays a role in oesophageal hypersensitivity, possibly modulated by psychological stress [31-34]. In ***chapter 5*** of this thesis we describe the effect of acute psychological stress on oesophageal acid perception.

The increased oesophageal sensitivity to acid, reflected by the development of reflux symptoms and/or complications, may be brought about by dilation of the intercellular spaces between adjacent epithelial cells. Dilated intercellular spaces (DIS) visualized with electron microscopy are found more frequently in GORD patients compared to healthy volunteers [35]. In theory, intraluminal acid may stimulate afferent neurons that can be reached more readily through these dilated intercellular spaces [36]. Although DIS is not a very specific sign, as 30% of healthy controls have signs of DIS as well, it is considered to be a marker of increased permeability of the oesophageal mucosa [37, 38].

Obese patients are more likely to have a hiatal hernia, oesophagitis, Barrett's oesophagus and reflux symptoms than patients with a normal body mass index (BMI) [39-41]. Several mechanisms can contribute to the development of reflux symptoms and complications in these patients, such as an increased incidence of TLOSRs and an increased intragastric pressure [42, 43].

Diagnosis

According to the Montreal definition, the diagnosis of GORD can be established by identifying mucosal damage, such as oesophagitis, or by identifying a causal relationship between symptoms and reflux episodes [5].

Reflux oesophagitis is defined endoscopically by visible breaks of the distal esophageal mucosa and can be classified according to the Los Angeles (LA) classification into 4 grades of severity: LA grade A to D, with grade D reflecting the most severe oesophageal injury [44]. It is unclear whether patients with various degrees of oesophageal damage have different types of reflux. This question is addressed in ***chapter 6*** of this thesis.

However, endoscopic lesions are found in only 15 to 20% of the patients with reflux symptoms, indicating that a substantial part of the patients with GORD do not have endoscopic abnormalities. Upper endoscopy is thus not a very sensitive tool for diagnosing GORD [9].

If endoscopy does not reveal reflux oesophagitis, the next step in order to diagnose GORD can be ambulatory oesophageal reflux monitoring. Oesophageal pH monitoring not only provides information on the magnitude of acidic reflux (expressed as the oesophageal acid exposure) but also on the relationship between reflux symptoms and reflux episodes. The latter is considered to be very important as the overlap in oesophageal acid exposure between patients with reflux symptoms and healthy controls is large [22, 45].

Several incidences can be used to express the relationship between symptoms and reflux episodes in patients with reflux symptoms. The symptom index (SI) and the symptom association probability (SAP) are the most frequently used [46, 47]. The SI is the percentage of symptoms preceded by a reflux episode within a 2-minute time window. When the SI is ≥50%, the patient's symptoms are considered to be caused by reflux. A disadvantage of this index is that it does not take the total number of reflux episodes into account. When a patient has many reflux episodes, this parameter can be ≥50% by mere chance. The SAP overcomes this problem because this parameter reflects the statistical likelihood that reflux symptoms are related to reflux episodes, considering both the number of reflux symptoms and number of reflux episodes.

Ambulatory 24-hour pH monitoring can be used to investigate the patient's reflux characteristics. Three types of pH electrodes are currently available to measure oesophageal pH: antimony electrodes, ion-sensitive field effect transistor (ISFET) electrodes and glass electrodes. In this thesis we compare these different types of pH electrodes under in vitro and in vivo conditions (***chapter 2***).

These days, oesophageal pH monitoring can be combined with intraluminal impedance monitoring. This technique allows detection of reflux episodes irrespective of their pH. Combined pH-impedance monitoring has shown to be a more accurate technique to assess the occurrence of reflux episodes and to investigate the relationship between symptom events

and reflux episodes [48-50]. Furthermore, this technique allows detection of movements of liquid and gas both in oral and in aboral direction. Therefore, impedance monitoring makes it possible to distinguish different reflux components such as liquid and gas. Also the number of swallows can be identified, and distinction can be made between normal swallows and swallows accompanied by air (air swallows) [51].

Using the pH recordings, each reflux episode identified in the impedance tracings can be classified as acid, weakly acidic or weakly alkaline reflux [52]. Since the detection of reflux episodes is pH-independent, combined pH-impedance monitoring can be performed during the use of acid secretion inhibitory therapy [53, 54].

Routine use in clinical settings is hampered by the lack of reliable software that is able to identify reflux episodes, as manual analysis is very time-consuming. In this thesis we assessed the accuracy of automated analysis software for detection of reflux episodes (***chapter 3***).

Therapy

Acid secretion inhibitory drugs are the mainstay of medical therapy for patients with reflux symptoms. Proton pump inhibitors (PPIs) are often prescribed both in the acute and chronic therapy of GORD, and are highly effective in the resolution of oesophagitis and reflux symptoms, both in approximately 80% of patients [55, 56].

In a proportion of patients, reflux symptoms are not adequately relieved during high-dose PPI therapy. The most likely cause of this treatment failure is an erroneous diagnosis of GORD. In an attempt to clarify the most helpful diagnostic approach in patients with reflux symptoms despite PPI therapy, we investigated the diagnostic yield of pH-impedance monitoring 'on' and 'off' PPI therapy (***chapter 4***).

At the present time, new medical treatments are investigated. Baclofen, a γ-amino-*n*-butyric acid ($GABA_B$) agonist, has been shown to decrease the number of TLOSRs and number of reflux episodes, but its use in clinical practice is hampered by significant neurological side effects, of which nausea and drowsiness are the most prevalent [57, 58]. Newer $GABA_B$ agonists, which are designed to work only peripherally and therefore have possibly less central side effects, are currently investigated [59, 60].

Another drug that inhibits TLOSRs and increases LOS basal pressure through an antagonism of the metabotropic glutamate receptor 5 (mGluR5) is currently investigated and also may have a role in clinical management of GORD [61, 62].

If medical therapy is inadequate or the patient does not want to take drugs lifelong, surgical therapy can be opted for. Surgical intervention has shown not only to decrease the number of reflux episodes, but also to reduce reflux symptoms and PPI use, and to increase quality of life [63-67]. Similar to other surgical interventions, this intervention also has its risks and

complications, of which post-surgery dysphagia is the most common [68]. Therefore, careful patient selection preoperatively is needed [69, 70].

For the time being, endoscopic antireflux procedures should be considered as experimental. Until today, no endoscpic procedure has proven to be more effective than the medical (PPI therapy) or surgery (laparoscopic Nissen fundoplication) gold standard [71-73].

BELCHING

Belching is a physiological phenomenon, occurring mainly after ingestion of meals or beverages, at a rate of 3-4 per hour. As described above, TLOSRs act as a protective mechanism to prevent the stomach from excessive dilatation. During TLOSRs air can escape from the stomach, and this is also known as normal gastric belching.

GORD patients or patients with functional dyspepsia often experience symptoms of excessive belching. It has been shown that they swallow air more often compared to healthy volunteers [74-76]. This excessive air swallowing leads to an increased number of TLOSRs during which air can escape from the stomach [77]. In this thesis we investigated the belching patterns and swallowing frequencies in GORD patients (***chapter 7 and 8***).

AEROPHAGIA

Patients who present with troublesome excessive belching as primary symptom are often believed to swallow air too frequently and are diagnosed with aerophagia, literally 'air eating', according to the Rome III criteria for functional gastroduodenal disorders [78].

In these patients, increased air swallowing frequencies and increased amounts of intragastric air appear not to be the cause of excessive belching. Instead, they exhibit another belching pattern, known as supragastric belching [79]. This typical belch pattern can be recognized using oesophageal impedance monitoring and is characterized by a rapid influx of air, followed by rapid air expulsion. These supragastric belches can occur in impressive numbers, reaching frequencies of 10-20 supragastric belches per minute. Interestingly, the frequency of supragastric belching can be influenced by attention and distraction, suggesting that this concerns a behavioural disorder [80]. In this thesis we investigated whether speech therapy can be of benefit in patients with excessive supragastric belching (***chapter 10***).

Whereas in many patients with excessive belching the term aerophagia is a misnomer, some patients really do swallow air more frequently. This may result in increased volumes of intestinal air. We investigated swallowing frequencies and belching patterns in patients with increased amounts of intestinal gas, visualized on plain abdominal radiograms (***chapter 9***).

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Ambulatory oesophageal pH monitoring: a comparison between antimony, ISFET, and glass pH electrodes

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ABSTRACT

Background & aim: Ambulatory oesophageal pH-impedance monitoring is a widely used test to evaluate patients with reflux symptoms. Several types of pH electrodes are available: antimony, ISFET and glass electrodes. These pH electrodes have not been compared directly, and it is uncertain whether these different types of pH electrodes result in similar outcome.

Methods: In an in vitro model the response time, sensitivity and drift of an antimony, ISFET and glass pH electrode were assessed simultaneously after calibration at 22°C and at 37°C. All measurements were performed at 37°C and repeated 5 times with new catheters of each type. Fifteen patients with reflux symptoms underwent 24-h pH monitoring off PPI therapy using antimony, ISFET and glass pH electrodes simultaneously.

Results: After calibration at 22°C, pH electrodes had similar response times, sensitivity and drift. In contrast to glass electrodes, antimony electrodes performed less accurate after calibration at 37°C than after calibration at 22°C. Calibration temperature did not affect ISFET electrodes significantly. During in vivo experiments, significant differences were found in acid exposure times derived from antimony (4.0±0.8%), ISFET (5.7±1.1%) and glass pH electrodes (9.0±1.7%).

Conclusion: In vitro, antimony and glass pH electrodes are affected by different buffer components and temperature, respectively. In vivo, significant differences between acid exposure times are obtained with antimony, ISFET and glass pH electrodes. ISFET electrodes produce stable in vitro measurements and result in the most accurate in vivo measurements of acid exposure time.

INTRODUCTION

Ambulatory oesophageal pH-impedance monitoring has shown to be a useful tool to quantify gastro-oesophageal reflux episodes and to assess a potential relationship between symptoms and reflux episodes [1, 2]. The most widely used parameter that is measured during 24-h pH monitoring is oesophageal acid exposure time. This parameter expresses the percentage of time with a pH below 4 traditionally measured at 5 cm above the upper border of the lower oesophageal sphincter (LOS). The acid exposure time has shown to be a good predictor of the outcome after anti-reflux surgery and is positively related to complications of reflux disease [3].

Several types of pH electrodes are currently available for intra-oesophageal pH monitoring. The use of glass pH electrodes for gastric acid measurements was first described in 1939 and the glass electrode is usually considered to be the most sensitive and stable electrode [4-6].

Unfortunately, glass pH electrodes are expensive and relatively large and they can not be combined with impedance electrodes in a single catheter.

Antimony pH electrodes are slimmer and less expensive compared to glass electrodes and can be easily combined with impedance monitoring. In laboratorium settings, the antimony catheter has been shown to be less accurate compared to glass electrodes [6]. Other studies show that with the correct thermal compensation algorithm, results obtained with antimony and glass electrodes show good agreement and differences do not affect the clinical outcome [7].

A third type of pH electrode is the Ion Sensitive Field Effect Transistor (ISFET) electrode. This electrode has shown to be reliable to assess luminal acidity [8, 9]. Like the antimony electrode, the ISFET electrode is small and can be combined with impedance electrodes in a single catheter. Besides that, several ISFET electrodes can be mounted on one catheter, enabling simultaneous pH measurements at multiple locations [10].

All three types of pH electrodes are currently widely available and used in clinical practice but a head-to-head comparison of the three catheters in vitro and in patients with symptoms of gastro-oesophageal reflux disease has not yet been carried out. The aim of our study was to compare the performance of antimony, ISFET and glass pH electrodes under in vitro and in vivo conditions.

METHODS

Materials

The three types of pH electrodes were compared in in vitro and in vivo studies. pH measurements were performed using glass electrodes (MIC Medical Instruments Corporation Solothurn, Solothurn, Switzerland), antimony electrodes (Phersaflex, Alpine Biomed, Fountain

Valley, California, USA) and ISFET electrodes (Unisensor AG, Attikon, Switzerland) simultaneously. The catheters were attached to each other with adhesive tape, taking care that the electrodes were located at the same level without shielding each other. In addition to a pH electrode the ISFET catheter had 8 ring electrodes enabling impedance recording from 6 segments.

The three pH catheters were attached to a single datalogger (Ohmega, MMS, Enschede, the Netherlands) which was customized for this purpose. All pH signals were stored using a sample frequency of 1 Hz. The impedance signals of the combined pH-impedance catheter were stored using a sample frequency of 50 Hz.

In vitro study

In order to simulate routine measurements, the catheters were calibrated at room temperature (22°C) using buffer solutions with pH 4.01 and 7.00 (Reagecon Diagnostics Limited, Shannon, Co. Clare, Ireland) before each measurement. All in vitro measurements were performed at 37°C. A temperature correction was used for the antimony electrode as advised by the manufacturer (-0.4 pH units applied to all pH values).

After calibration, the basic performance characteristics - response time, sensitivity and drift - were examined subsequently. Drift was assessed in a buffer solution with pH 4.01 and in artificial gastric juice (see below).

The in vitro measurements, set up to obtain response time, sensitivity and drift in artificial gastric juice, were repeated after calibration at 37°C in order to rule out a temperature effect.

Response time

The response time was evaluated by transferring the pH catheter between two buffer solutions with a known pH (pH 0.9 and 6.7 at 37°C). The response time was defined as the time needed for the reading from the pH electrode to reach 90% of its final value. Measurements were repeated 3 times and 5 different catheters of each type of pH electrode were examined.

Sensitivity

The sensitivity was assessed in 5 pH electrodes of each type over the pH range of 1.0 to 8.0 at 37°C using commercially available phosphate-free buffer solutions (Reagecon Diagnostics Limited, Shannon, Co. Clare, Ireland). The recorded pH values of the three types of electrodes were plotted against the pH of the test solution, recorded with a standard laboratory calomel pH electrode (InoLab® pH 720, WTW, Weilheim, Germany). The sensitivity of each type of pH electrode was derived from the slope of the graph using linear regression.

Drift

During a 24-h in vitro study, drift was assessed for 5 catheters of each type of pH electrode. After the calibration procedure, the catheters were immersed in a buffer solution with pH 4.01 at 37°C. Drift was defined as the difference between the pH value at the start and at the end of the 24-h recording period.

Drift in artificial gastric juice

To simulate the oesophageal environment during reflux episodes we fabricated artificial gastric juice that included bile contents [11, 12]. The artificial bile solution was diluted 20-fold and mixed with the artificial stomach solution. The final suspension (with CaCl2, KCl, NaCl, NaHCO3, Sodium azide, albumin, sodium taurocholate, egg yolk phosphatidylcholinecholesterol) was titrated using 1M HCl to pH 4.0.

After calibration, as described above, the catheters were immersed in the artificial gastric juice (at 37°C) during a 24-h recording period.

In vivo study

Patients and study protocol

Fifteen consecutive patients with reflux symptoms were recruited from the out-patients clinic of our department. The medical ethical committee of the University Medical Center Utrecht approved the protocol and informed consent was obtained from each subject.

All participants underwent ambulatory 24-h pH-impedance monitoring after cessation of acid secretory inhibiting therapy. Proton pump inhibitors and H_2-antagonists were discontinued 7 and 3 days prior to the ambulatory study, respectively.

Ambulatory pH-impedance monitoring

Ambulatory 24-h pH-impedance monitoring was carried out using antimony, ISFET and glass pH electrodes simultaneously. The three catheters were attached to each other in order to facilitate the introduction and to assure that the pH electrodes were located at the same level in the oesophagus at 5 cm above the manometrically located upper border of the LOS. Before introduction, all three catheters were calibrated at room temperature (22°C) using buffer solutions with pH 4.0 and 7.0.

The catheter carrying the ISFET pH electrode also contains 8 ring electrodes, allowing impedance recording from 6 segments (2-4 cm, 4-6 cm, 6-8 cm, 8-10 cm, 14-16 cm and 16-18 cm above the upper margin of the LOS).

During the measurement the patients were instructed to consume 3 meals and 4 beverages at fixed times. They were instructed to keep a diary in which they had to note these meal periods and periods that they spent in recumbent position.

Data analysis

All 24-h pH-impedance tracings were analyzed manually. Reflux episodes were detected using impedance tracings and classified according to previously published criteria into liquid reflux episodes, mixed liquid-gas reflux episodes and pure gas reflux episodes [1]. Liquid-containing reflux episodes were classified according to their pH into acid (with pH nadir below 4), weakly acid (with pH nadir between 4 and 7) and weakly alkaline (with pH above 7).

For each liquid-containing reflux episode the pH drop, pH nadir and acid clearance time (duration in seconds with a pH below 4.0) were derived from each type of pH electrode.

Oesophageal acid exposure time was expressed as percentage of time with a pH below 4. Excessive oesophageal acid exposure was defined as percentage of time with pH<4 >6% [13].

Statistical analysis

Data distribution was evaluated using the Kolmogorov-Smirnov test. The in vitro characteristics of each type of catheter were compared using a one-way ANOVA followed by Tukey's post hoc test. A paired Student *t*-test was used to compare different response times of each pH catheter. Comparisons between acid exposure times obtained by the 3 types of pH electrodes were made using a mixed-effects ANOVA with pH electrodes as fixed and patients as random factors. Differences between groups were analyzed using a Tukey's post hoc test. To compare percentages of acid and non-acid reflux episodes identified with the three types of pH electrodes a Chi-square test was used followed by a logistic regression. Throughout the manuscript data is presented as mean ± SEM or median and interquartile range. P-values <0.05 were considered to be statistically significant.

RESULTS

In vitro study

Response time

The 90% pH values were reached with all pH electrodes. The mean time needed to reach 90% of the pH of the buffer solution is shown in table 1 for each type of pH electrode, both after calibration performed at 22ºC and at 37ºC.

The glass pH electrodes when calibrated at 37ºC showed slightly longer response times during transfer from neutral to acid buffer solution compared to the antimony and ISFET electrodes ($p<0.05$). Other differences between antimony, ISFET and glass electrodes were not statistically significant.

All pH electrodes showed prolonged response times of transfer from acid to neutral buffer solutions compared to the transfer from neutral to acid buffer solutions, although not all differences were statistically significant (antimony electrode after calibration at 22ºC ($p=0.74$)

and at 37°C (p=0.06) and the glass electrode after calibration at 22°C (p=0.15)). No statistically significant differences were identified in response times between calibration at 22°C or 37°C for each type of pH electrode.

		Response time (s) Neutral → acid 90%	**Response time (s) Acid → neutral 90%**
Calibration at 22°C	Antimony	6.3 ± 5.1	8.8 ± 4.0
	ISFET	1.4 ± 0.4	4.8 ± 0.8
	Glass	1.5 ± 0.4	7.4 ± 3.2
Calibration at 37°C	Antimony	0.7 ± 0.1	7.2 ± 2.5
	ISFET	1.0 ± 0.2	3.1 ± 0.4
	Glass	1.6 ± 0.2	2.7 ± 0.2

Table 1 Response time (mean ± SEM): defined as number of seconds needed to reach 90% of the final pH value after transferring antimony, ISFET and glass pH electrodes from neutral (buffer pH 7) to acid buffer (buffer pH 1) solutions and vice versa (both after calibration at 22°C and at 37°C)

Sensitivity

After calibration at 22°C, the sensitivity (expressed as pH response/pH unit measured with laboratory pH electrode) was comparable between the three types of electrodes (antimony: 0.99 pH/pH unit; ISFET: 1.10 pH/pH unit; glass: 1.04 pH/pH unit) (figure 1A).

When the pH electrodes were calibrated at 37°C all three types of electrodes had similar sensitivity as well (antimony: 1.06 pH/pH unit; ISFET: 1.06 pH/pH unit; glass: 1.02 pH/pH unit) although measurements with the antimony electrode resulted in significantly lower pH values (figure 1B).

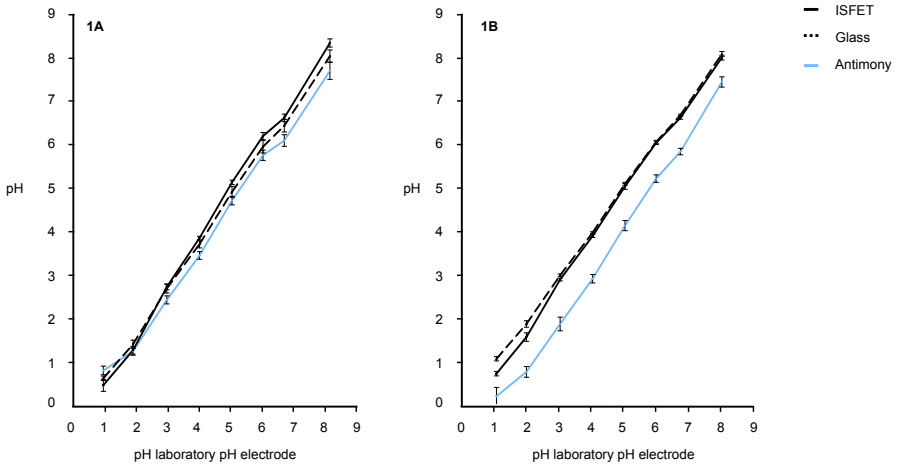

Figure 1 pH of buffer solutions at 37°C obtained by antimony, ISFET, glass and laboratory pH electrodes after calibration at 22°C (1A) and after calibration at 37°C (1B). Y-axis: measured pH with antimony, ISFET, glass and laboratory electrode. X-axis: pH value of the buffer solutions obtained by the laboratory pH electrode.

Drift buffer solution with pH 4 after calibration at 22°C

Drift did not differ between antimony (0.2 ± 0.1 pH-units/24-h), ISFET (0.0 ± 0.0 pH-units/24-h) and glass electrodes (0.1 ± 0.1 pH-units/24-h)(p=0.18) during the 24-h measurement in the buffer solution (figure 2A).

Drift in artificial gastric juice after calibration at 22°C

No statistically significant differences were found between drift of the antimony electrode (0.3 ±0.2 pH units/24-h), ISFET (-0.1±0.1 pH units/24-h) and glass pH electrodes (0.1±0.1 pH units/24-h)(p=0.12). The use of glass pH electrodes resulted in significantly lower pH values compared to laboratory pH electrode (figure 2B).

Drift in artificial gastric juice after calibration at 37°C

Figure 2C shows the pH values of the artificial gastric juice both at the start and end of the measurement after calibration at 37°C. The antimony electrode had more drift (0.5 ± 0.2 pH units/24-h) compared to both ISFET and glass electrodes (0.1±0.0 pH units/24-h and 0.0 ± 0.0 pH units/24-h, respectively) ($p<0.05$) and showed significantly lower pH values compared to the laboratory pH electrode.

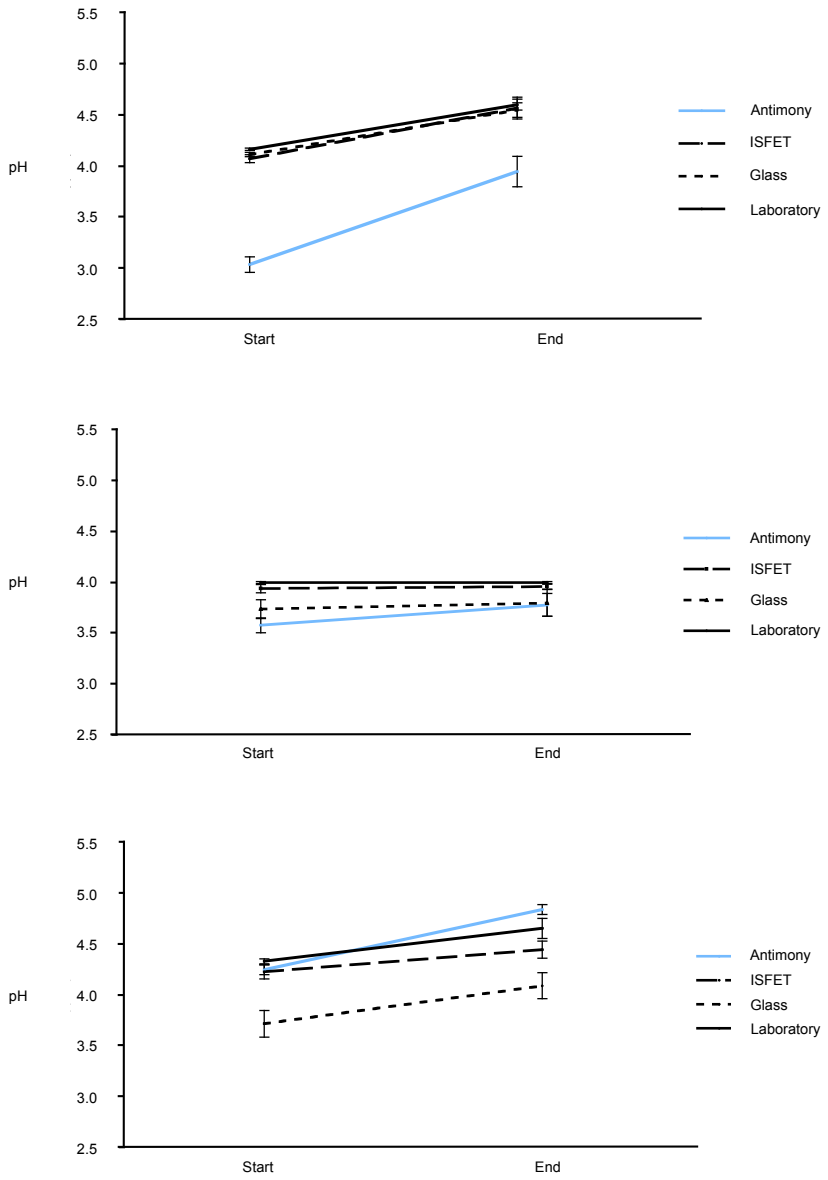

Figure 2 Average (± SEM) pH values of antimony, ISFET and glass pH electrode at the start and end of the 24-h measurement in a buffer solution with pH 4.00±0.01 at 37°C after calibration at 22°C (A), and in artificial gastric juice after calibration at 22°C (B) and at 37°C (C)

In vivo study

Fifteen consecutive patients (age: 47.2 ± 15.2y (SD); 7 females) underwent an ambulatory 24-h pH-impedance measurement. One patient was excluded from the analysis due to failure of the hardware.

Glass pH electrodes resulted in significantly higher acid exposure times compared to the antimony and ISFET and pH electrodes ($p<0.05$)(table 2). Figure 3 shows individual acid exposure times obtained by the three types of pH electrodes. Four patients with an increased acid exposure were identified using antimony electrode, 6 patients using ISFET electrode and 7 patients using glass electrode.

Acid exposure time (%)	**Antimony**	**ISFET**	**Glass**
Total	4.0 ± 0.8	5.7 ± 1.1	9.0 ± 1.7 *
Upright	5.1 ± 1.2	6.7 ± 1.6	11.2 ± 2.2 *
Supine	2.0 ± 1.1	3.4 ± 1.7	4.9 ± 2.2

Table 2 Acid exposure times (total and in upright and in supine position) obtained by antimony, ISFET and glass pH electrodes. Acid exposure time: percentage of time with pH below 4 at 5cm above the upper border of the LOS. * Statistically significant differences between glass and ISFET and glass and antimony electrodes ($p<0.05$)

In table 3 the numbers of acid, weakly acidic and weakly alkaline reflux episodes are shown. Reflux episodes were more often classified as acid reflux episodes using the glass pH electrode compared with antimony and ISFET electrode ($p<0.05$).

Mean pH drop during reflux episodes did not differ between the three types pH electrodes (antimony: 2.0 ± 0.2 pH units; ISFET: 1.8 ± 0.2 pH units; glass: 2.1 ± 0.3 pH units).

Glass pH electrodes resulted in significantly increased acid clearance times (glass: 54 ± 11 s) compared to both antimony and ISFET pH electrodes (antimony: 39 ± 7 s; ISFET: 45 ± 7 s; $p<0.05$).

During liquid-containing reflux episodes glass electrodes resulted in significantly lower pH nadirs (glass: 3.1 ± 0.3) compared to antimony and ISFET electrodes (antimony: 4.4 ± 0.3; ISFET: 3.9 ± 0.3). Differences in pH nadir between antimony and ISFET electrodes were also statistically significant ($p<0.05$).

		Antimony	ISFET	Glass
Liquid	Acid	6 ± 2	7 ± 2	9 ± 3
	Weakly acidic	10 ± 3	9 ± 3	7 ± 2
	Weakly alkaline	1 ± 0	0 ± 0	0 ± 0
Mixed liquid-gas	Acid	13 ± 3	13 ± 2	18 ± 4
	Weakly acidic	10 ± 3	12 ± 3	6 ± 1
	Weakly alkaline	1 ± 0	0 ± 0	0 ± 0

Table 3 Numbers of liquid-containing reflux episodes (liquid and mixed liquid-gas) classified into acid, weakly acidic and weakly alkaline reflux episodes according to their pH, measured with antimony, ISFET and glass pH electrodes. Using glass electrodes, liquid-containing reflux episodes were more frequently classified as acid reflux episodes.

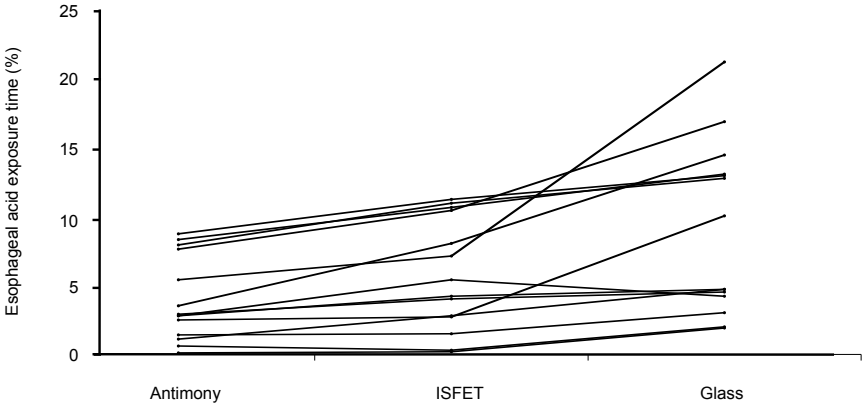

Figure 3 Individual acid exposure times (percentage of time with pH<4 at 5cm above the upper border of the LOS) simultaneously recorded with antimony, ISFET and glass pH electrodes. LOS: lower oesophageal sphincter

DISCUSSION

This is the first study that compared the in vitro and in vivo characteristics of the currently available pH electrodes for intra-oesophageal pH monitoring simultaneously. Several studies have investigated the characteristics of the different types of pH electrodes in vitro, but it was unclear to what in vitro differences led to clinically significant differences [6-8, 14].

In this study, we showed that antimony, ISFET and glass pH electrodes resulted in clinically significant differences in acid exposure times and numbers of acid, weakly acidic and weakly alkaline reflux episodes.

In addition, marked differences were found between the three types of pH electrodes during the in vitro experiments we performed. The antimony electrode measured lower pH values compared with the laboratory pH electrode and ISFET and glass pH electrodes when immersed in buffer solutions at 37°C after calibration at 22°C (figure 1A). From these results one would expect to find the lowest pH values and largest acid exposure times during the 24-h ambulatory measurements in patients using antimony electrodes. Surprisingly, this is not the case. Despite the applied temperature correction, antimony electrodes resulted in significantly lower acid exposure times (thus higher pH values) compared to ISFET and glass electrodes during the ambulatory measurement (table 2). These findings confirm a previously published study in which antimony pH electrodes resulted in decreased oesophageal acid exposure times compared to glass pH electrodes [5].

Most strikingly, after calibration at 37°C measurements with antimony electrodes in vitro resulted in very low pH values. These in vitro experiments were performed at the same temperature as during the calibration process, indicating that a temperature effect could not have influenced these results. Instead, despite the use of phosphate-free buffer solutions, the antimony electrode was probably affected by the different components of buffer solutions. Previous publications have shown that certain components, such as phosphate, influence the reliability of antimony electrodes [15]. In addition, in artificial gastric solution antimony electrodes had significantly more drift (0.5 ± 0.2 pH units/24-h) compared to ISFET and glass electrodes after calibration at 37°C. Apparently, antimony electrodes were affected by both temperature and components of buffer solutions or stomach contents. After calibration at 22°C, the effect of temperature and the buffer components were possibly counteracting each other, and resulting in relatively normal pH values when measuring at 37°C.

At the other end, in vivo measurement using a glass pH electrode resulted in the largest oesophageal acid exposure times. We showed that a temperature effect is responsible for the lower pH values during the in vitro experiments after calibration at 22°C, as differences between the laboratory and glass pH electrode were very small and could be disregarded when calibrated at 37°C. These results indicate that the temperature during the calibration

process certainly plays a role and suggest that for in vivo studies glass electrodes should be calibrated at 37ºC.

Interestingly, the ISFET pH electrodes performed very well during the in vitro experiments, with regard to response times, linearity (pH response per pH unit) and drift, both after calibration at 22ºC and 37ºC. In line with previous publications, absolute differences with laboratory pH electrodes were small, only at the low extremes (pH buffer 1 and 2) pH values were significantly lower than the laboratory pH electrode [8, 9].

In conclusion, we have shown that ambulatory pH monitoring performed with antimony, ISFET and glass pH electrodes simultaneously results in clinically significant differences in oesophageal acid exposure times. These results underscore the importance of catheter-type specific normal values in clinical pH testing. In contrast with glass and antimony pH electrodes, ISFET pH electrodes perform reliably both after calibration at 22ºC and at 37ºC.

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Computer analysis of 24-h esophageal impedance signals

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ABSTRACT

Background: Ambulatory 24-h pH-impedance monitoring has become the standard technique for detection of gastroesophageal reflux. Manual analysis of the 24-h pH-impedance recordings requires expertise and is time-consuming. The aim of our study is to assess the accuracy of newly developed software for detection of reflux episodes.

Study: 24-h esophageal impedance recordings obtained from 10 patients were manually analyzed by 3 investigators. Liquid-containing reflux episodes and their proximal extent were scored. A consensus between the 3 investigators was used as a gold standard. Computer analysis using dedicated software was performed and the results were compared with the results of the consensus agreement. In addition, 24-h impedance tracings of 60 consecutive patients with reflux symptoms were analyzed both manually by one investigator and using computer software. The number of reflux episodes and the results of symptom association analysis obtained by the human and computer analysis software were compared.

Results: The consensus meeting resulted in a total of 625 reflux episodes. The mean sensitivity and the percentage of true-positives of analysis by individual investigators was 89 ± 1% and 94 ± 1%, respectively. Automated analysis had a sensitivity of 73 ± 4% and a proportion of true-positive reflux episodes of 62 ± 8%. Symptom association analysis performed by the computer and a human observer showed concordant results in 83% of the patients.

Conclusions: Although not as good as manual analysis by experts, computer analysis can be a helpful tool to identify reflux episodes and to assess the relationship between reflux episodes and symptoms.

INTRODUCTION

In patients with symptoms of gastroesophageal reflux disease (GERD) ambulatory 24-h esophageal pH-impedance monitoring has become the standard technique to detect episodes of gastroesophageal reflux. Several studies have shown that combined pH-impedance monitoring is superior to pH monitoring alone with regard to detection of reflux episodes and yield of symptom association analysis [1-3].

The analysis of impedance tracings requires expertise and is time-consuming when conducted manually. With increased use of combined pH-impedance monitoring in both research and clinical settings, the need for reliable software is growing.

Although GERD patients in general have more reflux events than healthy subjects, a large overlap exists in numbers of reflux episodes between different groups of GERD patients [4]. More importantly, in patients without endoscopic lesions, the diagnosis of GERD is based on symptoms and their temporal relationship with reflux episodes [5]. Therefore, not only the numbers of reflux events, but also the association between reflux episodes and symptoms is of particular interest.

A previous study showed that available software for automated detection of reflux episodes results in overestimation of the number of reflux episodes and provides inaccurate symptom association analysis in 20% of the patients [6].

The aim of our study was to assess the accuracy of newly developed software for detection of liquid-containing reflux episodes and for symptom association analysis, as compared with the results with manual analysis.

METHODS

Patients

Data obtained from 60 consecutive patients with reflux symptoms during a 24-h pH-impedance measurement were used in this study. All patients underwent ambulatory 24-h pH-impedance monitoring after the cessation of PPI therapy for at least 7 days [3].

Esophageal pH-impedance monitoring

Prior to the ambulatory 24-h pH-impedance measurement, all patients underwent a stationary manometry to locate the upper border of the lower esophageal sphincter (LES).

A combined pH-impedance catheter (Versaflex, Alpline Biomed, Fountain Valley, CA) with 6 impedance recording segments and 1 antimony pH electrode was used for the ambulatory measurement. The catheter was placed with the pH electrode located at 5 cm above the

upper border of the LES. The impedance recording segments were located 2–4 cm, 4–6 cm, 6–8 cm, 8–10 cm, 14–16 cm, and 16–18 cm above the upper margin of the LES.

The combined pH-impedance catheter was attached to a digital datalogger (Ohmega, MMS, Enschede, The Netherlands) in which the pH and impedance signals were stored at a sample frequency of 1 Hz and 50 Hz, respectively [7].

During the measurement patients were instructed to consume 3 meals and 4 beverages at fixed times, and to note these periods in a diary. In addition, patients were asked to report their symptoms during the measurement by pressing the event marker button on the digital datalogger and to note the time of onset and to describe the nature of the symptom in the diary.

Analysis of reflux events

Three experienced investigators (G.J.H., A.J.B., A.J.S.) manually analyzed the 24-h impedance recordings of 10 consecutive patients independently. The investigators were blinded to the pH recordings. The experts were instructed to identify all liquid-containing reflux episodes and to determine their proximal esophageal extent using the impedance tracings. Reflux episodes were defined using previously described criteria [8].

Since the detection of the reflux episodes by the automatic analysis software is based on the variations of impedance signals and not on variations of the pH, the detection of reflux episodes was conducted independently of their pH.

After the individual analysis, the results of the three investigators were compared. During a consensus meeting, all identified reflux episodes were discussed and a consensus on all recorded liquid-containing reflux episodes was achieved. This consensus was used as the gold standard.

After the consensus was reached, automatic analysis software (Version 8.11a, July 28th 2008 build 1744, MMS, Enschede, The Netherlands) analyzed the 24-h impedance recordings of the 10 patients. The onset and proximal extent of the reflux episodes were evaluated.

The sensitivity was calculated as the percentage of the number of identified reflux episodes by the observer divided by the total number of reflux episodes according to the gold standard.

Since it was not possible to score true-negative reflux episodes, specificity could not be calculated. Instead, the proportion of reflux episodes correctly scored by the observer was calculated (percentage of true-positives).

Symptom association analysis

The 24-h pH-impedance recordings of all 60 patients were analyzed manually by one experienced investigator (G.J.H.). The total number of liquid-containing reflux episodes was assessed and symptom association analysis was performed using the symptom index (SI) [9] and symptom association probability (SAP) [10]. A symptom was considered to be related to a

reflux episode when a reflux episode was followed by the symptom within a 2-minute time window [11].

Afterwards, automated detection of the liquid-containing reflux episodes of the 24-h recordings was performed using the analysis software, and the SI and SAP were calculated automatically.

Hereafter, reflux episodes identified by computer analysis were verified and removed when the investigator considered the event as false-positive, and the number of true reflux episodes and results of the symptom association analysis were assessed once more.

Statistical analysis

Throughout the manuscript data is presented as mean ± SEM or as median and interquartile range. Normality was tested using the Kolmogorov-Smirnov test. A Kendall's W test was calculated to compare the proximal extent scored during the consensus agreement with proximal extent scored by investigators and automatic analysis software. A paired Student's *t*-test was used to compare numbers of reflux episodes identified by the investigator and automatic analysis software. Comparisons between manual and automatic analyses were performed using Chi-square tests. Differences were considered statistically significant when $p<0.05$.

RESULTS

Manual analysis

In the 24-h impedance tracings of 10 consecutive patients (3 male, 7 female; mean age ± SD: 47.6 ± 12.2y) a consensus agreement was reached regarding the presence and extent of reflux episodes. The 24-h recordings had a mean recoding time of 21 hour and 26 minutes excluding meal periods.

Every reflux episode detected by each investigator was evaluated during the consensus agreement. A total number of 712 impedance events were recognized by one or more investigators, of which 625 events were considered to be reflux episodes by consensus. This was used as the gold standard. Of these reflux episodes, 78% were identified by all 3 investigators, 15% were found by 2 investigators and 8% by only one of the investigators.

Individual results of the investigators are shown in table 1. The investigators had a mean sensitivity of 89 ± 1%. Of all identified reflux episodes, 94 ± 1% was a true reflux episode according to the gold standard.

Reflux episodes had a mean proximal extent of 7 ± 2 cm. Proximal extent scored by the investigators corresponded well with proximal extent scored during the consensus agreement (Kendall's W values: 0.716, 0.811 and 0.745).

		Sensitivity (%)	True positives (%)
Observer	1	90 ± 2	93 ± 4
Observer	2	92 ± 1	92 ± 3
Observer	3	85 ± 3	94 ± 3
Computer		73 ± 4	62 ± 8

Table 1 Sensitivity and percentage of true-positives of the detection of reflux episodes by individual investigator and by computer analysis.

Automated analysis

The computer analysis detected a total of 781 impedance events of which 114 impedance events were marked as unknown. Of these 781 impedance events 464 were regarded as true reflux episodes with the gold standard. The automated analysis software had a mean sensitivity of 73 ± 4% and 62 ± 8% of the identified reflux episodes were true positive according to the gold standard (table 1). Reflux episodes had a proximal extent of 5 ± 2cm. The proximal extent scored by the automated analysis showed moderate agreement with the proximal extent scored by the investigators during the consensus agreement (Kendall's W value: 0.598). The proportion of reflux episodes reaching various proximal extents scored by the observers during the consensus agreement and automated analysis are shown in figure 1.

Symptom association analysis

In order to investigate whether the differences that are obtained with automated analysis resulted in differences in symptom association analysis, the 24-h recordings of 60 patients (30 male, 30 female; age: 47.4 ± 12.6y) were analyzed, with a net recording time of 21 hour and 24 minutes ± 59 minutes (SD).

The number and type of reflux episodes identified by the observer and by the automated analysis software is shown in table 2. The automated analysis software detected similar numbers of liquid-containing reflux episodes compared to the observer. After removal of false-positive reflux episodes by the investigator, automated analysis resulted in a significantly lower number of reflux episodes ($p<0.05$).

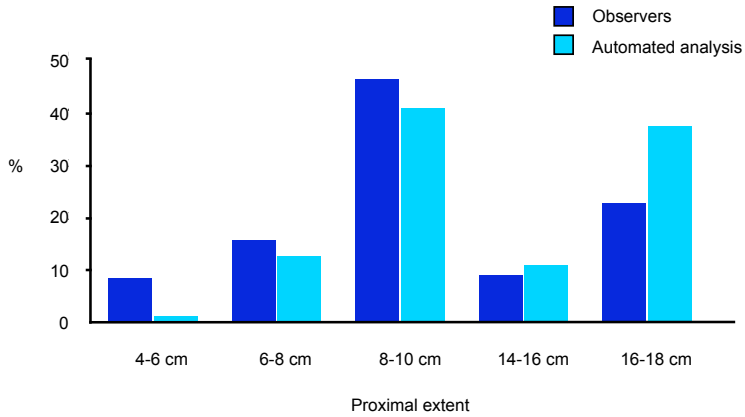

Figure 1 The percentage of reflux episodes reaching different proximal extents is shown for both the consensus agreement and automated analysis. Proximal extent in cm from the upper border of the lower esophageal sphincter (LES).

	Manual analysis	Computer analysis - before exclusion	Computer analysis- after exclusion
Total	60 ± 5	57 ± 6	43 ± 5*
Acid	42 ± 5	36 ± 5*	32 ± 4*
Weakly acidic	17 ± 1	20 ± 2	11 ± 1*
Weakly alkaline	1 ± 0	1 ± 0	1 ± 0

Table 2 Numbers of liquid-containing reflux episodes in 60 patients identified manually and using automated analysis before and after removal of false-positive reflux episodes (mean ± SEM). * $p<0.05$ compared with manual analysis

The patients experienced 7 (3-12) symptoms during the measurement, of which 4 (1-8) symptoms were reflux-related, as they occurred in a 2-minute time window following a reflux episode.

Symptom association analysis performed by the investigator resulted in the identification of 34 patients with a positive SI and 34 patients with a positive SAP (table 3).

Using the automated analysis software, 22 patients with a positive SI and 26 patients with a positive SAP were identified. Expelling false-positive reflux episodes and verification of the

unknown reflux events detected by the automated analysis software did not result in the identification of extra patients with a positive SI or positive SAP.

Concerning the SAP, concordant results were found in 50 out of the 60 patients (83%). According to the observer and the automated analysis software 25 patients had a positive SAP and 25 patients had a negative SAP. Discordant results were obtained in the remaining 10 patients. Nine patients had a positive SAP according to the investigator and a negative SAP according to the automated analysis software. Only 1 patient had a positive SAP according to the automated analysis software and had a negative SAP according to the observer.

Twelve patients with a positive SI according to the investigator did not have a positive SI using the automated analysis software.

Table 3A		**Computer analysis**		
		SAP -	SAP +	
Manual analysis	SAP -	25	1	26
	SAP +	9	25	34
		34	26	60

Table 3B		**Computer analysis**		
		SI -	SI +	
Manual analysis	SI -	26	0	26
	SI +	12	22	34
		38	22	60

Table 3 Results of symptom association analysis using the SAP (A) and SI (B) performed manually by a human observer and using analysis software. Performing analysis manually, more patients with a positive SAP (A) and SI (B) were identified compared to using the automated analysis ($P<0.05$).

DISCUSSION

Combined 24-h pH-impedance monitoring has shown to be a very sensitive and reliable method not only to measure gastroesophageal reflux but also to assess the relationship between symptoms and reflux episodes [2, 8, 12]. However, routine use of impedance tracings in clinical practice is hampered by the time-consuming analysis of the impedance signals.

In the present study we assessed the accuracy of newly developed software for automated detection of liquid-containing reflux episodes from 24-h impedance tracings.

A consensus agreement of 3 experienced investigators was used as a gold standard interpretation and was used to calculate the accuracy of the automatic analysis of impedance signals. The acidity of the identified reflux episodes during the consensus agreement was not taken into account, since reflux episodes are detected using impedance signals only, thus pH-independently.

The majority of reflux episodes was identified by all observers (78%). In the other 22%, one or two investigators failed to identify the reflux episode, or one was not convinced that the event was a reflux episode. Although strict criteria for the recognition of reflux episodes are available, during manual analysis a human observer may decide that the criteria do not apply to a specific impedance event and that the criteria should be overruled.. This reflects the reality that some reflux episodes are difficult to identify or that their presence can be questionable.

At first sight, the total numbers of reflux episodes identified by automated analysis appeared to be correct (table 2). Automated analysis resulted in significantly decreased numbers of reflux episodes after removal of false-positive reflux episodes, resulting in an underestimation of the total number of reflux episodes.

In the clinical setting, results of symptom association analysis may be of more importance than the number of reflux episodes. Despite the fact that a significant number of reflux episodes was not detected by the computer analysis, results of the symptom association analysis (SAP) were concordant in 83% of the patients. In the majority of the other 17%, patients had a positive relationship between symptoms and reflux episodes according to the investigator only. This is probably due to the lower sensitivity of the analysis software for reflux detection, as the results of the symptom association analysis are not affected by removal of false-positives.

Our results are comparable with a previous study using another software program for automatic reflux detection (Autoscan of Bioview® software) [6]. The authors concluded that automatic analysis results in an overestimation of the number of reflux episodes and disagreement of the SI (using a 5-minute time window) in 20% of cases. Unfortunately, only the number of reflux events were compared and reflux episodes identified by automated

analysis were not verified by the investigators. These issues may have led to an overestimation of the level of agreement between the computer analysis and manual analysis.

In conclusion, we have shown that automated analysis software can be a helpful tool in assessing the relationship between reflux episodes as detected by esophageal impedance monitoring and symptoms. However, symptom association analysis performed by computer analysis showed discordant results compared to manual analysis in nearly 20% of the cases. Therefore, for the time being, manual analysis remains the standard to assess number of reflux episodes and their relationship with reflux symptoms.

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Esophageal pH-impedance monitoring in patients with therapy-resistant reflux symptoms: 'on' or 'off' proton pump inhibitor?

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ABSTRACT

Background: In patients with PPI-resistant symptoms ambulatory 24-h pH-impedance monitoring can be used to assess whether a relationship exists between symptoms and reflux episodes. Until now, it is unclear whether combined pH-impedance monitoring in these patients should be performed on or off PPI.

Methods: Thirty patients with symptoms of heartburn, chest pain and/or regurgitation despite PPI b.i.d. underwent ambulatory 24-h pH-impedance monitoring twice, once on PPI and once after cessation of the PPI for 7 days. The order of the measurements was randomized. Reflux episodes were identified and classified as acid, weakly acidic or weakly alkaline reflux. In addition, the symptom association probability (SAP) was calculated for each measurement.

Results: The total number of reflux episodes and proximal extent were not affected by PPI therapy. On PPI, there were fewer acid reflux episodes (49 ± 34 off PPI vs. 20 ± 25 on PPI) while more weakly acidic reflux episodes were identified (24 ± 17 off PPI vs. 48 ± 31 on PPI). Symptom association analysis identified 15 and 11 patients with a positive SAP in the measurement off and on PPI, respectively, the difference in yield of the SAP not being statistically significant. Eight of the 19 patients who had no symptoms or a negative SAP during measurement on PPI had a positive SAP off PPI therapy. In contrast, only 4 patients with a positive SAP on PPI were missed in the measurement off PPI therapy.

Conclusions: In order to demonstrate or exclude GERD in patients with PPI-resistant symptoms, ambulatory 24-h pH-impedance monitoring should preferably be performed after cessation of PPI therapy because this approach seems to offers the best chance to assess a relationship between symptoms and reflux episodes.

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a very common disorder in the western world; 10-20 % of the population experience symptoms of heartburn or regurgitation at least once a week [1, 2]. According to the Montreal classification, GERD is a condition that develops when reflux from the stomach into the esophagus causes symptoms and/or mucosal damage [3]. Patients who seek medical care are usually treated satisfactorily with proton pump inhibitors (PPI) by general practitioners. However, some patients have persistent symptoms of heartburn, regurgitation and chest pain despite acid-suppressive therapy. These patients are often referred to a gastroenterologist for further diagnostic workup and treatment.

The most important reason for treatment failure is an erroneous diagnosis of GERD [4]. Several functional disorders can be misinterpreted as GERD, and treatment with a PPI is unlikely to resolve the symptoms in these patients.

Secondly, several studies have shown that weakly acidic reflux episodes can also cause symptoms of heartburn and regurgitation [5-7]. Since PPIs do not reduce the number of reflux episodes but only change the acidity, weakly acidic reflux episodes can persist as the cause of symptoms in patients who use PPIs [5, 8].

Another potential cause of treatment failure is insufficient inhibition of gastric acid production by the PPI therapy. This may occur either because of limited effectiveness of the PPI itself [9] or because patients are not compliant to the therapy. Finally, because acid secretion will never be fully inhibited by PPI treatment, a few remaining acidic reflux episodes may be the cause of the patients' symptoms, despite adequate acid-suppressive therapy.

Ambulatory 24-h monitoring of gastroesophageal reflux has been shown to be very helpful in assessing a potential relationship between symptoms and reflux episodes. This used to be done with esophageal pH monitoring after subjects had discontinued their acid-suppressive therapy for several days, because with this technique only acid reflux episodes could be detected. With the recently developed impedance monitoring however, reflux episodes are detected independently of their acidity. This method has been shown to be a sensitive and reproducible method to assess the number and type of reflux episodes and to investigate the relation between symptoms and reflux episodes [7, 10, 11]. In patients with PPI-resistant symptoms, it is unclear if 24-h pH-impedance monitoring should be performed on or off PPI therapy. Therefore, the aim of our study was to compare the yield of 24-h pH-impedance monitoring off and on PPI therapy in GERD patients with PPI-resistant symptoms.

METHODS

Subjects

For this multicenter randomized cross-over study patients with typical reflux symptoms (heartburn, regurgitation and/or chest pain) despite PPI therapy b.i.d. were included. Patients with a history of esophageal or gastric surgery were excluded. All patients were recruited from the population of patients at the St. Antonius Hospital Nieuwegein, the Universital Medical Center Utrecht, and the Central Military Hospital in Utrecht, the Netherlands. Written informed consent was obtained from all subjects before the start of the study and the protocol was approved by the local medical ethical committees.

Study Protocol

Prior to the ambulatory measurements all patients underwent upper endoscopy on PPI therapy. Combined ambulatory 24-h pH-impedance monitoring was performed twice on two separate occasions with an interval varying between one and four weeks. In a randomized order, one measurement was performed after cessation of PPI for 7 days, while the other measurement was performed on double dose PPI therapy (b.i.d.). Before the first measurement, the lower esophageal sphincter (LES) was located by stationary manometry in order to position the pH-impedance catheter correctly.

Esophageal impedance and pH monitoring

For the ambulatory measurements a combined pH-impedance catheter was used (VersaFlex, Alpine Biomed, Fountain Valley, California, USA). This catheter contains 8 ring electrodes for impedance measurements which enable recording from 6 2-cm long segments, and a single antimony pH electrode.

After detection of the LES by manometry, the combined pH-impedance catheter was placed with the antimony pH electrode 5 cm above the upper margin of the LES. Impedance recording segments were located at 2-4 cm, 4-6 cm, 6-8 cm, 8-10 cm, 14-16 cm and 16-18 cm above the upper margin of the LES.

Impedance and pH data were stored in a digital datalogger (Ohmega, MMS, Enschede, The Netherlands) using a sample frequency of 50 Hz and 1 Hz respectively.

During both measurements, patients were instructed to have three meals and four beverages at fixed times. The patients kept a diary in which periods of ingestion and periods spent in recumbent position were noted. Furthermore, the patients were instructed to press the event marker button on the digital datalogger whenever they experienced a symptom and to describe the nature and onset of their symptoms in the diary. To obtain a representative measurement with symptoms, patients were encouraged to maintain their normal daily activities.

Data analysis

Analyses of the 24-h recordings were carried out after the second measurement was completed and all recordings were analyzed manually.

Reflux episodes were defined as a fall in impedance of ≥ 50% of baseline impedance that moved in retrograde direction in the two distal impedance sites. Reflux episodes were considered to have reached the proximal esophagus when the impedance fall reached the two most proximal recording segments located at 14-18 cm above the LES. Reflux episodes were classified as mid-esophageal reflux episodes if they reached the middle recording segments (6-10 cm above the LES) and as distal reflux episodes when they reached only the two distal recording segments (2-6 cm above the LES).

Reflux episodes were classified as acid when the pH dropped below 4, and as weakly acidic when pH nadir was between 4 and 7. Weakly alkaline reflux was defined as a reflux episode during which the pH did not drop below 7 [12]. Periods of meal consumption were excluded from the analysis. Acid exposure time was calculated as the percentage of time with pH below 4. Excessive acid exposure was defined as the percentage of time with pH<4 >6.0% off PPI therapy [13] while on therapy an acid exposure time of >1.6% was defined as indicative of inadequate acid suppression [14].

Symptom Analysis

Symptom-reflux association analysis was carried out to investigate the relationship between the occurrence of reflux episodes and symptoms. Only typical reflux symptoms (heartburn, chest pain and regurgitation) were used for further analysis [15]. Reflux episodes were considered symptomatic when a symptom episode occurred in the 2-minute time window preceding the reflux episode [16]. The symptom index (SI) and the symptom association probability (SAP) [17] were calculated. When the SAP was ≥95%, the patients' symptoms reflux episodes were considered to be related to gastroesophageal reflux.

Statistical Analysis

The parametric data are presented as mean ± standard deviation and comparisons were performed using the paired Student *t* test. The non-parametric data are presented as median (interquartile range) and were compared using the Wilcoxon Signed Ranks test. The McNemar test was used to compare the results of the symptom association analysis between both measurements. A P value <0.05 was considered to be statistically significant.

RESULTS

Patients

Thirty-seven patients were enrolled in the study. Three patients were excluded because of failure of the hardware, and 4 patients were not willing to undergo the second measurement. Thirty patients (mean age: 46.5y; range: 19.1-71.8y; 20 male) underwent both measurements successfully.

All 30 patients underwent upper endoscopy on PPI therapy prior to the 24-h pH-impedance measurements. According to the Los Angeles classification, grade C esophagitis was present in two patients and grade B esophagitis in one patient. Four patients had a hiatal hernia larger than 3 cm.

Reflux Parameters

As expected, the total number of reflux episodes was not influenced by PPI therapy (73 ± 33 off PPI vs. 69 ± 35 on PPI, (p=0.341)) (figure 1). In addition, the percentage of reflux episodes reaching the proximal (p=0.271), mid- (p=0.824) or distal esophagus (p=0.241) did not change significantly between the two measurements (figure 2).

During the measurement on PPI the number of acid reflux episodes was lower (49 ± 34 off PPI vs. 20 ± 25 on PPI (p<0.001)) while more weakly acidic reflux episodes were found (24 ± 17 off PPI vs. 48 ± 31 on PPI (p<0.001)) in comparison with the measurement off PPI. Compared to the number of acid and weakly acidic reflux episodes, the number of weakly alkaline reflux episodes was very low (2 ± 3 off PPI vs. 2 ± 3 on PPI) and was not affected by the PPI therapy (p=0.958). As expected, the percentage of time with pH below 4 was less during PPI therapy (off PPI: 5.0% (2.0-14.2%); on PPI: 1.1% (0.2-6.3%); p=0.007)(table 1).

Acid exposure time (%)	Off PPI	On PPI	P value
Total	5.0 (2.0 - 14.2)	1.1 (0.2 - 6.3)	<0.01
Upright	7.5 (2.6 - 13.8)	1.5 (0.3 - 8.9)	<0.01
Supine	0.5 (0.0 - 6.8)	0.0 (0.0 - 1.3)	0.12

Table 1 Median (interquartile range) acid exposure time (% of time with pH<4) off and on PPI therapy in total and in upright and supine position

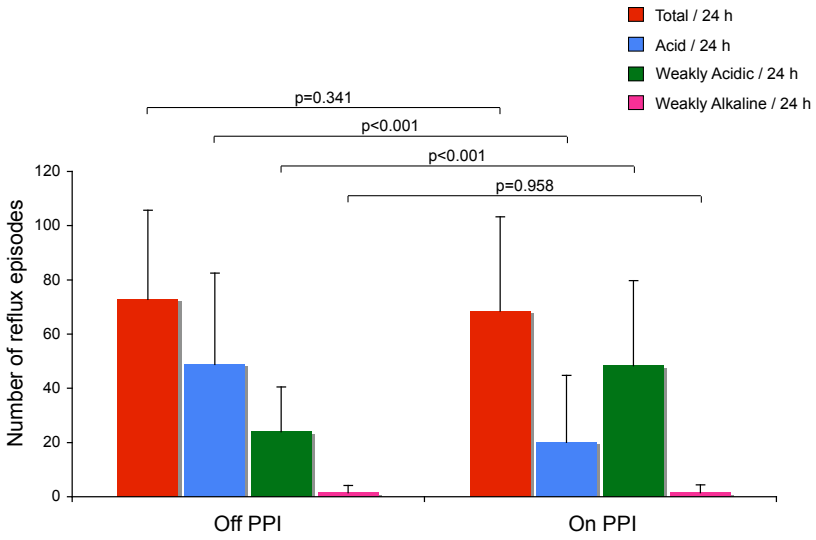

Figure 1 Total numbers and numbers of acid, weakly acidic and weakly alkaline reflux episodes are shown, off and on PPI therapy (mean + SD)

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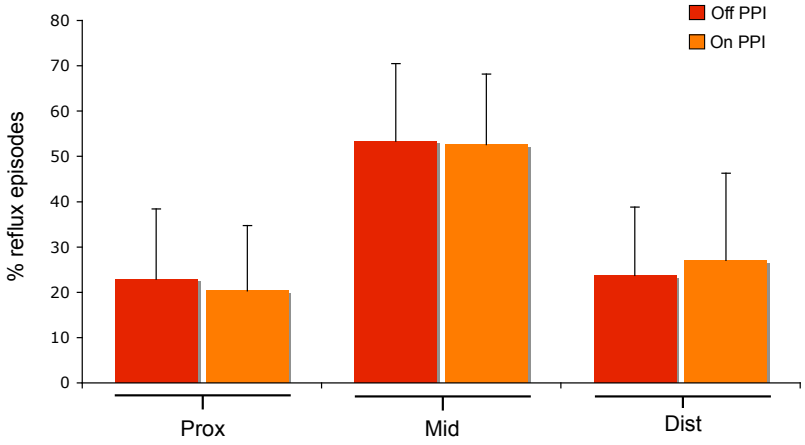

Figure 2 Percentage of reflux episodes reaching the proximal, middle and distal esophagus off and on PPI therapy.

Symptom Association Analysis

The individual results of the symptom association analysis are shown in table 2 and summarized in table 3. During the measurement off PPI 2 patients reported no reflux symptoms. In the remaining 28 symptomatic patients symptom analysis was performed and this resulted in the identification of 13 patients with a negative SAP and 15 patients with a positive SAP. Nineteen patients had a positive SI and 9 patients had a negative SI.

During the measurement on PPI 7 patients were asymptomatic. In the remaining 23 patients a negative SAP was found in 12 patients and a positive SAP in 11 patients (figure 3). Twelve patients had a positive SI and 11 patients had a negative SI.

The result of symptom association analysis was concordant for both measurements in only 15 patients. Seven patients had a negative SAP and 7 patients had a positive SAP in both measurements. One patient was asymptomatic during both measurements (table 3).

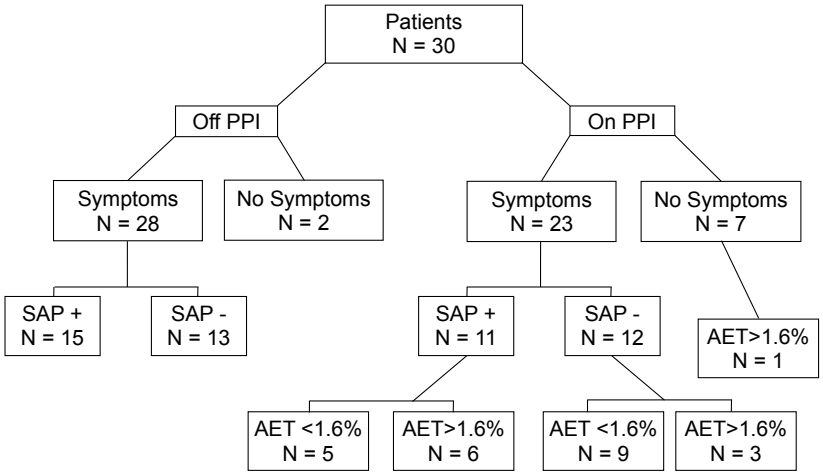

Figure 3 Results of symptom association analysis off and on PPI therapy and relationships with adequately (% of time with pH<4 <1.6%) and inadequately suppressed acid exposure (% of time with pH<4 ≥1.6%) during PPI therapy. SAP: symptom association probability; SAP+: SAP≥95%; SAP-: SAP<95%; AET: acid exposure time.

	AET off PPI (%)	AET on PPI (%)	SI off PPI (%)	SI on PPI (%)	SAP off PPI (%)	SAP on PPI (%)
1	6.3	0.0	+	No Sx	+	No Sx
2	2.2	0.4	+	No Sx	-	No Sx
3	7.9	11.4	+	+	+	+
4	0.1	0.2	0	-	0	-
5	1.3	0.1	0	-	0	-
6	33.3	1.2	+	+	+	+
7	3.3	10.3	-	0	-	0
8	1.8	4.1	0	+	0	+
9	5.1	0.7	No Sx	+	No Sx	+
10	11.0	0.1	+	+	+	+
11	5.0	0.3	0	0	0	0
12	5.4	1.4	+	No Sx	-	No Sx
13	2.6	1.4	+	0	-	0
14	10.2	0.2	+	0	+	0
15	1.6	1.1	+	No Sx	-	No Sx
16	4.9	1.3	+	+	+	+
17	15.1	11.7	+	+	+	+
18	2.0	9.2	-	+	-	+
19	2.9	1.1	+	0	+	0
20	21.2	13.7	+	No Sx	+	No Sx
21	18.2	30.6	+	0	-	0
22	0.3	0.1	No Sx	No Sx	No Sx	No Sx
23	4.2	5.1	+	+	+	+
24	0.0	0.0	-	0	+	0
25	21.0	0.2	+	0	+	0
26	24.7	10.7	+	+	+	+
27	3.3	0.0	0	+	0	+
28	1.1	0.2	-	-	-	-
29	13.9	0.8	+	No Sx	+	No Sx
30	17.3	5.3	+	+	+	-

Table 2 Individual results of acid exposure times and symptom association analysis off and on PPI. AET: Acid exposure time (excessive acid exposure time is defined as the percentage of time with pH<4 >6.0% 13 or >1.6% 14 off or on PPI therapy, respectively). SI: symptom index (+: ≥ 50%, -: < 50%; 0: no symptoms related to reflux; No Sx: no symptoms) SAP: symptom association probability (+: ≥ 95%, -: < 95%; 0: no symptoms related to reflux; No Sx: no symptoms

		On PPI			
		No Sx	SAP-	SAP+	
Off PPI	No Sx	1	0	1	2
	SAP-	3	7	3	13
	SAP+	3	5	7	15
		7	12	11	30

Table 3 Concordance of the results of symptom association analysis off and on PPI therapy (number of patients). No Sx: asymptomatic during 24-hour monitoring; SAP-: negative symptom association probability; SAP+: positive symptom association probability

In the other 15 patients the results of the two measurements were discordant. Seven patients were asymptomatic during one of the measurements (1 patient off PPI and 6 patients on PPI); as a result no SAP could be calculated. In these patients the other measurement identified 4 patients with a positive and 3 patients with a negative SAP. In the remaining 8 patients a different SAP was calculated for both measurements: 5 patients had a positive SAP in the measurement off PPI and a negative SAP in the measurement on PPI and 3 patients had a positive SAP in the measurement on PPI and a negative SAP off PPI. Of these 3 patients 1 patient had a hiatus hernia and esophagitis (grade C). This patient had an excessive acid exposure time only during the measurement on PPI. The other patients had normal upper endoscopy. One patient had normal esophageal acid exposure during both measurements and the other patient had an excessive acid exposure only during the measurement on PPI.

Eight patients without a positive SAP on PPI (3 asymptomatic and 5 patients with a negative SAP) had a positive SAP in the measurement off PPI. In contrast, during the measurement off PPI 4 patients who had a positive SAP on PPI were missed and did not have a good relation between symptoms and reflux episodes (3 patients) or were asymptomatic (1 patient) during this measurement. The differences in yield of the SAP between both measurements (table 3) were not statistically significant (p=0.118).

In order to investigate a potential sequence effect, the results of the first measurement were compared with the second measurement, regardless of PPI use. In the first measurement 16 out of 34 (47%) patients had a positive SAP and in the second measurement 13 out of 30 (43%) patients had a positive SAP, indicating that the results are not likely to be influenced by a sequence effect.

Three out of the four patients who were not willing to undergo the second measurement had a positive SAP, and were all measured after cessation of PPI therapy. The other patient, who was measured on PPI, had a negative SAP.

When the pH recordings were analyzed independently of the impedance tracings, symptom association analysis resulted in a positive SAP in 12 patients during one or both measurements. Eleven and 7 patients had a positive SAP for acid reflux in the measurement off and on PPI, respectively. Six patients had a positive SAP for acid reflux during both measurements. Thus pH-impedance monitoring had a higher yield than pH monitoring alone since it allowed identification of 4 additional patients with a positive SAP off PPI and 4 additional patients with a positive SAP on PPI therapy.

In the measurements off PPI excessive acid exposure (defined as % of time with pH<4 greater than 6.0%) was found in 12 patients. In the measurement on PPI 10 patients had excessive acid exposure (defined as % of time with pH<4 greater than 1.6) (table 4). Six patients had an excessive acid exposure during both measurements.

Of the 18 patients with a normal acid exposure off PPI, 2 patients had a positive SAP identified on pH monitoring alone. The remaining 16 patients were asymptomatic (n=2) or had a negative SAP (n=14). Of these 16 patients 4 patients were asymptomatic, 4 had a negative SAP and 4 patients had a positive SAP during the combined pH-impedance measurement on PPI.

The number of symptoms and number of symptoms related to reflux was significantly lower during the measurement on PPI (3 (1-6) and 1 (0-3), respectively) compared to the measurement off PPI (5 (2-10)(p=0.004) and 2 (1-6)(p=0.010), respectively).

During PPI therapy, the number of heartburn episodes related to reflux decreased significantly (1 (0-4) off PPI vs. 0 (0-1) on PPI, p=0.006) while the numbers of chest pain episodes and regurgitation were not significantly different (p=0.755 and p=0.507, respectively). In the measurement on PPI a high proportion (71.9%) of heartburn episodes related to reflux was still preceded by an acid reflux episode (figure 4).

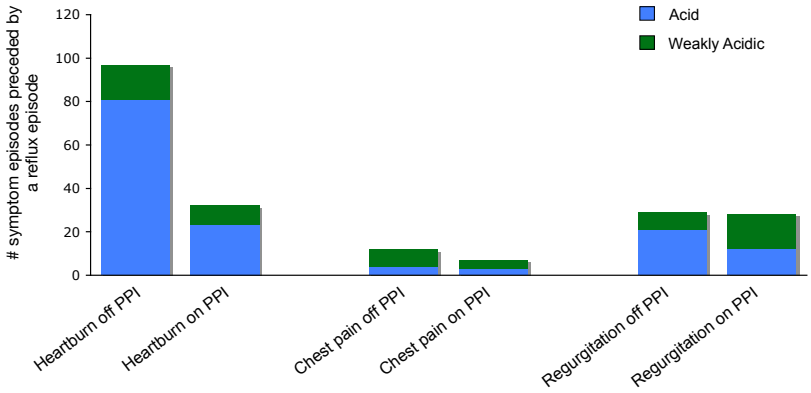

Figure 4 Number of symptom episodes (heartburn, chest pain, regurgitation) preceded by acid and weakly acidic reflux episodes off and on PPI therapy.

	30 patients	
Acid Exposure	**Off PPI**	**On PPI**
Normal	18	20
No Sx	2	6
SAP-	12	9
SAP+	4	5
Excessive	12	10
No Sx	0	1
SAP -	1	3
SAP +	11	6

Table 4 Number of patients with normal and excessive acid exposure off and on PPI therapy. Excessive acid exposure is defined as % of time with pH<4 more than 6.0% 13 and 1.6% 14 off and on PPI, respectively. No Sx: asymptomatic; SAP-: negative symptom association probability; SAP+: positive symptom association probability.

DISCUSSION

This is the first study in which ambulatory esophageal 24-hour pH-impedance monitoring both on and off PPI therapy was carried out in one and the same group of patients with PPI-refractory reflux symptoms. The specific aim of our study was to determine which of the two approaches is optimal in the work-up of these patients, measurement after temporary interruption of PPI therapy, or measurement while PPI treatment is continued. The former approach allows one to measure baseline esophageal acid exposure, a traditional and robust measure of the severity of gastroesophageal reflux. The latter approach offers the possibility to assess the adequacy of PPI treatment in terms of remaining esophageal acid exposure. Both approaches allow assessment of the temporal association between symptom episodes and reflux events.

In our opinion, the first and most important question that needs to be addressed in patients with PPI-refractory reflux symptoms is whether or not their symptoms are brought about by reflux. Therefore, in the interpretation of the results of our study we considered the yield of symptom association analysis as the primary study outcome. Since the SAP takes both the numbers of reflux events as the number of symptoms into account, we relied primarily on the SAP to distinguish between patients with a good relation between symptoms and reflux episodes and those without.

We showed that performing both measurements has the highest yield as far as the identification of patients with a positive SAP is concerned. Of course, this is not the most desirable approach in the majority of patients.

Our observation that 50% of the patients had a positive SAP off PPI and 37% on PPI therapy is in accordance with previously published data. In a study with 24-h pH-impedance monitoring in 168 patients on PPI, 39% of the patients had a positive SAP [18]. Zerbib et al. [19] performed a symptom association analysis off and on PPI therapy in two separate patient groups: 79 patients were studied off PPI and 71 patients on PPI therapy. Fifty-two percent of the patients off PPI and 31% of the patients on PPI therapy had a positive SAP, which suggests a higher yield of the ambulatory reflux monitoring after cessation of PPIs.

A higher proportion of our patients was asymptomatic during the measurement on PPI (6.7% off and 23.3% on PPI therapy). This, again, is in agreement with findings made by Zerbib et al. [19]. Obviously, the absence of symptoms during the measurement on PPI led to a decreased yield of the symptom association analysis. Two possible explanations for the lack of symptoms during the measurement on PPI therapy can be proposed. Firstly, patients could be more compliant to the PPI therapy during the study. Secondly, the patients' recollection of symptoms occurring despite PPI may not be correct. We chose not to exclude the patients who were asymptomatic in one of the two measurements for the analysis, because this reflects the situation in daily practice best.

In addition, almost half of the symptomatic patients with a negative SAP on PPI had a positive SAP in the measurement off PPI therapy. In contrast, only 4 of the 15 patients without a positive SAP off PPI had a positive SAP during PPI therapy. The higher proportion of patients with a positive SAP off PPI therapy is largely due to the lower incidence of symptom episodes during PPI therapy, since the number of reflux episodes and the proportion of symptoms related to reflux were the same in both measurements.

Among the symptom episodes scored, episodes of heartburn decreased during PPI therapy, while the frequencies of regurgitation and chest pain were not influenced by the PPI therapy. This is in accordance with the observation that heartburn is more commonly provoked by acid reflux [5]. Regurgitation and chest pain appear to be less acid-dependent.

Endoscopy did not contribute to the diagnostic work-up of GERD as it revealed esophagitis in only 3 patients. A negative endoscopy has low diagnostic value for GERD, especially when performed on PPI therapy.

The total number of identified reflux episodes was comparable with previous data from our group [20-22] but was high compared to findings of other groups [18, 19]. An explanation may be that our patients are explicitly encouraged to do their normal daily activities and not to avoid food and beverages that elicit symptoms.

An argument in favor of a measurement off PPI therapy is the possibility to evaluate the severity of naïve esophageal acid exposure, which is a parameter that predicts the response to anti-reflux surgery [23]. This parameter can be useful when anti-reflux surgery is considered.

Ten patients had an acid exposure during PPI therapy of more than 1.6% [14], indicating that acid secretion was not adequately inhibited. Some of these patients may have an increased metabolism of the PPI by the cytochrome systems in the liver [9], others may not have been not fully compliant to the therapy. Only 6 of the 11 patients with a positive SAP on PPI therapy had an insufficiently suppressed acid exposure. Five patients had a positive SAP despite adequate acid-suppressive therapy.

In agreement with previous studies, this study confirms the increased yield of combined pH-impedance monitoring compared to classic pH monitoring [19, 20]. In 4 of the 15 patients the addition of impedance monitoring was necessary to obtain a positive SAP off PPI therapy. In the measurement on PPI therapy the addition of impedance monitoring to pH monitoring increased the number of patients with a positive SAP from 7 to 11. In 4 of the 16 patients without a positive SAP and with a normal acid exposure time during pH monitoring off PPI, a positive SAP was found during the combined pH-impedance measurement on PPI.

It should be stressed that observed differences in yield of symptom association analysis between both measurements were not statistically significant, likely due to the relatively small sample size. The lack of concordance between both measurements is indicative of the fact that GERD varies day-by-day. Since the measurement off PPI resulted in the highest yield of the symptom association analysis, we consider this approach the most desirable. In patients

in whom the refluxogenic origin of the symptoms has been established before, and in whom there is doubt about the effectiveness of the PPI therapy, 24-h pH-impedance monitoring on PPI therapy can provide information about the degree of acid suppression that is obtained.

In conclusion, no statistically significant difference in yield of symptom association analysis between both approaches was found. In our opinion, to exclude or confirm GERD as the cause of symptoms in patients with PPI-resistant symptoms, ambulatory 24-h pH-impedance monitoring should preferably be performed after cessation of PPI therapy. This approach offers the best chance to assess the relationship between symptoms and reflux events in these patients. When reflux has been identified as the likely cause of the symptoms measurement of remaining esophageal acid exposure during PPI treatment becomes more meaningful.

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Does acute psychological stress increase perception of oesophageal acid?

5

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ABSTRACT

Background & Aim: GORD patients often report an increase of their reflux symptoms during stressful situations. The aim of this study was to assess the influence of acute psychological stress on oesophageal acid perception.

Methods: In 15 healthy volunteers and 10 GORD patients with a positive symptom-reflux association an oesophageal acid perfusion test was performed, once with and once without the presence of an acute psychological stressor (IQ test). The order of the measurements was randomized. The time from onset of the acid infusion to first acid perception, discomfort and pain was noted. Blood pressure was measured in order to assess the effect of the stress task.

Results: In healthy volunteers, the time to first perception (control task: 617 ± 174s vs. stress task: 561 ± 162s), discomfort (control task: 969 ± 158s vs. stress task: 940 ± 151s), or pain (control task: 1393 ± 122s vs. stress task: 1366 ± 121s) did not differ significantly between both measurements. In GORD patients, no significant differences between both measurements were found either in time to first perception (control task: 63 ± 26s vs. stress task: 43 ± 15s), discomfort (control task: 153 ± 44s vs. stress task: 249 ± 62s) or pain (control task: 558 ± 139s vs. stress task: 633 ± 118s). Systolic blood pressure rose significantly during the stress task in both healthy volunteers (6 ± 1 mmHg) and GORD patients (9 ± 2 mmHg).

Conclusion: Neither in healthy volunteers nor in GORD patients, the acute psychological stress induced by an IQ test increased oesophageal acid perception. The observed increase in systolic blood pressure shows that the experimental stressors were effective.

INTRODUCTION

Patients with gastro-oesophageal reflux disease (GORD) often report an association between an increase of their reflux symptoms and stressful circumstances. Several studies have investigated the relationship between symptoms of gastro-oesophageal reflux and psychological stress. Naliboff et al. [1] showed that the presence of a severe sustained life stress in the previous 6 months predicted increased heartburn in the following months, which implies that chronic stress plays a role.

Other studies showed that acute psychological stress, induced by laboratory stress tasks, increased subjective ratings of anxiety and reflux symptoms but did not lead to an increase in objective reflux parameters (acid exposure time or number of reflux episodes) [2-4].

A possible explanation for the increase in symptoms without an increase in acid exposure is induction of oesophageal hypersensitivity to gastro-oesophageal reflux during periods of acute stress.

Barlow and Orlando introduced the concept of dilated intercellular spaces (DIS) as a possible explanation for increased oesophageal hypersensitivity [5]. Theoretically, intraluminal acid can diffuse more easily through epithelium with dilated intercellular spaces and stimulate afferent neurons. In an animal model, Farré et al. found an increase in intercellular space after exposure to a laboratory stressor [6].

Recently Fass et al. showed that an auditory stressor increased heartburn sensation and intensity after oesophageal acid infusion [7]. The increased perception of reflux symptoms after sleep deprivation is another argument for central modulation of perception of gastro-oesophageal reflux [8].

These studies suggest a role of hypersensitivity to gastro-oesophageal reflux during periods of acute psychological stress. The main question to be addressed in this study is whether an acute psychological stressor influences perception of acid in the distal oesophagus in healthy volunteers and GORD patients.

METHODS

Subjects

Fifteen healthy volunteers without symptoms or history of GORD were recruited by means of advertisements. In addition, 10 patients with gastro-oesophageal reflux disease with a positive association between symptoms and reflux episodes (SAP≥95%) were recruited from the population of GORD patients attending the out-patient Gastroenterology Clinic of the University Medical Center Utrecht, The Netherlands.

Smoking and alcohol consumption was prohibited on the day before and the day of the measurements. GORD patients were allowed to take their acid secretion inhibitory drugs. Participants who had undergone surgery of the stomach or oesophagus were excluded.
The medical ethical committee of the University Medical Center Utrecht approved the study protocol and informed consent was obtained from every participant before the start of the study.

Study protocol
All subjects underwent an acid perfusion test twice, once without and once with an acute psychological stressor. The order of the measurements was randomized. A modified Raven Advanced Progressive Matrices IQ Test was used as a psychological stressor as described below [9, 10]. A video documentary served as a control task. Before introduction of the catheters all subjects filled in a validated Dutch translation of the State-Trait Anxiety Inventory (STAI) questionnaire regarding baseline anxiety [11, 12] levels on both occasions (figure 1).
Systolic and diastolic blood pressure and heart rate were measured with a non-invasive blood pressure monitor (Accutorr Plus, Danica Datascope) using an arm cuff (WelchAllyn). Before introduction of the manometry and pH catheter, blood pressure and heart rate were measured twice at 10-minute interval. During the acid perfusion and mental stress test, blood pressure and heart rate were recorded at 2.5-minute intervals.
Both measurements were performed within a 1-week interval and at the same time of day.

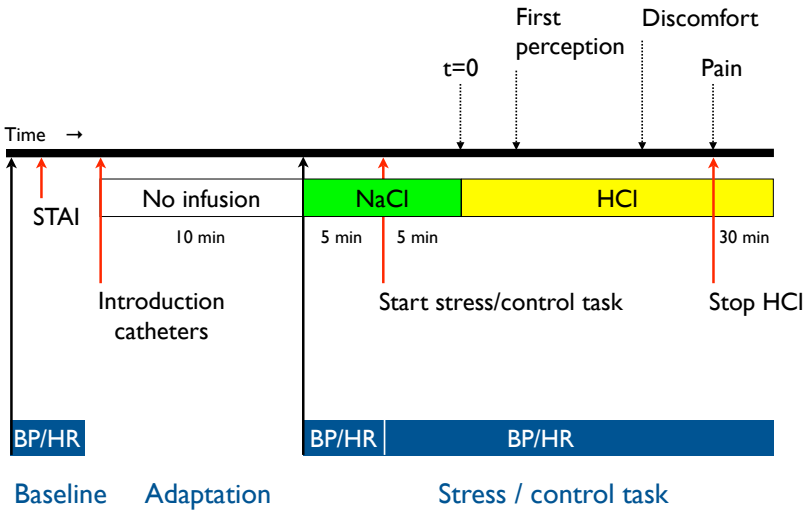

Figure 1 Overview of the study design. BP: Blood pressure; HR: heart rate; STAI: State Trait Anxiety Inventory questionnaire; t=0: start acid infusion

Acid perfusion test

After completion of the questionnaire, a water-perfused (0.45ml/min) 8-channel silicone rubber sleeve catheter was introduced (DentSleeve International Ltd, Mississauga, Ontario, Canada) with an outer diameter of 3.5 mm. The catheter was placed with the sleeve straddling the lower oesophageal sphincter (LOS) and with the distal oesophageal side-hole positioned at 5 cm above the upper margin of the LOS. After placement of the manometry catheter, a pH catheter was introduced with the pH electrode located at 5 cm above the upper border of the LOS.

After correct positioning of the catheters, water perfusion of the manometric assembly was discontinued and the distal oesophageal side-hole of the manometry catheter was attached to the infusion pump for saline or acid infusion.

An adaptation period of 10 minutes preceded the actual acid perfusion test. After this adaptation period, saline was infused in the distal oesophagus for 10 minutes, followed by hydrochloric acid (0.1 N) for 30 minutes or until the subject experienced pain. Both saline and hydrochloric acid were instilled at a rate of 8 ml/min controlled by an automatic pump (IVAC 560 Volumetric Pump, Rhys Int. Ltd, Bolton, UK).

The control or stress task was started after 5 minutes saline infusion and was aborted at the end of the acid infusion (figure 1).

Subjects were asked to report the first sensation of heartburn, discomfort and pain. When subjects experienced pain, acid infusion was discontinued immediately and the measurement was aborted. After every elapsed minute during the acid perfusion test, patients were asked whether their sensation changed.

The time from start of acid infusion (marked by a pH drop to pH<1.0) necessary to provoke first perception, discomfort and pain were noted.

To blind the participant for start of infusion, the acid infusion pump was placed behind the chair and participants were not informed about the start of acid infusion. Both the stress and control task started 5 minutes before the start of acid infusion.

Stress and control task

A modified Advanced Progressive Matrices Raven IQ test was used as a psychological stress task. This modified IQ test has previously shown to be an effective psychological stressor [9]. The Raven Matrices were presented at a personal computer and each problem, a multiple choice test of abstract reasoning, was shown for only 10 seconds. In this short period the subjects had to select the correct solution from the alternatives shown. In addition, to increase stress response during the test, four 3-second beeps were delivered, immediately before some of the more difficult matrix problems. Subjects were told before the start of the measurement that the computer tracked their performance and would beep when the subject's performance fell below the average of previous participants. In reality, the sound

delivery was pre-programmed and did not depend on performance. The beeps were delivered at equal intervals, and were the same for each subject.
After the last measurement, participants were debriefed and they were told that no IQ score was obtained and beeps were pre-programmed, independent of their performance.
As a control task a documentary was shown (Coral seas, Blue Planet, BBC, UK) using a TV and DVD player.

Data analysis
Distribution of all parameters was assessed using the Kolmogorov-Smirnov test. The state and trait anxiety scores and time (in seconds) needed to reach first perception, discomfort or pain thresholds were compared using a paired Student's *t*-test.
For both measurements, differences in blood pressure and heart rate were calculated as the mean blood pressure/heart rate during the acid perfusion test minus the blood pressure/heart rate during saline infusion. A paired Student's *t*-test was used to determine whether the differences in blood pressure and heart rate were statistically significant during saline and acid perfusion.
To investigate whether results were influenced by a significant sequence effect, times necessary to provoke first perception, discomfort and pain were compared by order of the measurements, regardless which task was presented.
Comparisons between healthy volunteers and GORD patients were performed using an independent t-test.
To investigate a possible relationship between STAI-DY scores and differences in time needed to reach first perception, discomfort or pain during both measurements, a Pearson's correlation coefficient was calculated.
Statistical analysis was performed using SPSS 11.0 for Mac OS X (version 11.0.4; SPSS Inc., Chicago, Il). Data was expressed as mean ± SEM. P values <0.05 were considered to be statistically significant.

RESULTS

Healthy volunteers

STAI Questionnaire

All fifteen healthy volunteers (mean age: 37y; range: 20-63y, 7 female) completed both measurements. Healthy volunteers had a trait anxiety score of 28 ± 1 (STAI-DY2). State anxiety levels did not differ between both measurement days (STAI-DY1 control: 31 ± 2 vs. STAI-DY1 stress: 29 ± 1; p=NS).

Blood pressure and heart frequency

Systolic and diastolic blood pressures and heart rate did not change significantly after the start of the video documentary (systolic blood pressure: -1 ± 1 mmHg; diastolic blood pressure: 0 ± 1 mmHg; heart rate: -1 ± 1 bpm; p=NS). In contrast, after the start of the IQ test, systolic blood pressures were significantly elevated (6 ± 1 mmHg; $p<0.05$) compared to blood pressures before the start of the IQ test. Diastolic blood pressures and heart rate were not significantly affected by the IQ test (0 ± 1 mmHg and 1 ± 2 bpm; p=NS).

Acid perfusion test

From the onset of acid infusion to the end of the measurement (acid infusion discontinued because of pain or after 30 minutes) oesophageal pH was <4 during 100% of time.

During the video documentary 2 of the 15 subjects did not feel the infused acid at all, 4 subjects did not experience discomfort, and 7 subjects did not experience pain after 30 minutes of acid infusion. During the IQ test, first perception was not reached in 1 subject, discomfort was not reached in 4 and pain was not reached in 6 subjects after 30 minutes of acid infusion.

There was no difference between the stress task and video conditions in time necessary to reach first perception (video: 617 ± 174 s vs. stress task: 561 ± 162 s; p=0.71), discomfort (video: 969 ± 158 s vs. stress task: 940 ± 151 s; p=0.84), or pain (video: 1393 ± 122 s vs. stress task: 1366 ± 121 s; p=0.80). Individual results (control vs. stress task) are depicted in figure 2.

The difference in time needed to reach first perception was related to the differences in time needed to reach discomfort ($p<0.01$, r=0.67) and pain thresholds (p=0.01, r=0.64).

No statistically significant relationships were found between the STAI-DY scores (STAI-DY1 and STAI-DY2) and differences in time necessary to reach first perception, discomfort and pain thresholds during control and stress task.

No evidence was found for a significant sequence effect that could influence the time needed to reach first perception, discomfort or pain thresholds.

GORD patients

STAI Questionnaire

Ten patients (age: 50y, range: 30-69y, 3 female) completed both measurements. GORD patients had a trait anxiety score of 36 ± 3 (STAI-DY2). State anxiety levels did not differ between both measurements (STAI-DY1 score video: 35 ± 3 vs. STAI-DY1 score stress task: 37 ± 2).

Blood pressure and heart frequency

Similar to healthy volunteers, changes in systolic (-3 ± 2 mmHg, p=NS) and diastolic (-1 ± 1 mmHg, p=NS) blood pressure and heart rate (-1 ± 1 bpm, p=NS) before and during the video documentary were not statistically significant. During the stress task, both systolic and diastolic blood pressures were elevated (9 ± 2 mmHg, p=0<0.01; 4 ± 2 mmHg, p=0.03) compared to before the start of the stress task. Heart rate did not differ significantly before and after the start of the IQ test (-2 ± 2 bpm; p=NS).

Acid perfusion test

Similar with healthy volunteers, oesophageal pH dropped below 4 during the entire acid infusion period. In all GORD patients oesophageal acid infusion led to first perception, discomfort and pain. No statistically significant differences were found for either time to first perception (video: 63 ± 26 s vs. stress task: 43 ± 15 s; p=0.40), discomfort (video: 153 ± 44 s vs. stress task: 249 ± 62 s; p=0.26) or pain (video: 558 ± 139 s vs. stress task: 633 ± 118 s; p=0.72). Individual results of time needed to reach first perception, discomfort or pain thresholds during both measurements are shown in figure 2.

No statistically significant correlation was found between differences in time during the control and stress task needed to reach first perception, discomfort and pain thresholds.

In accordance with healthy volunteers, no significant relationships were found between STAI-DY scores and differences in time needed to reach first perception, discomfort or pain thresholds between the control and stress task.

No evidence was found for a significant sequence effect.

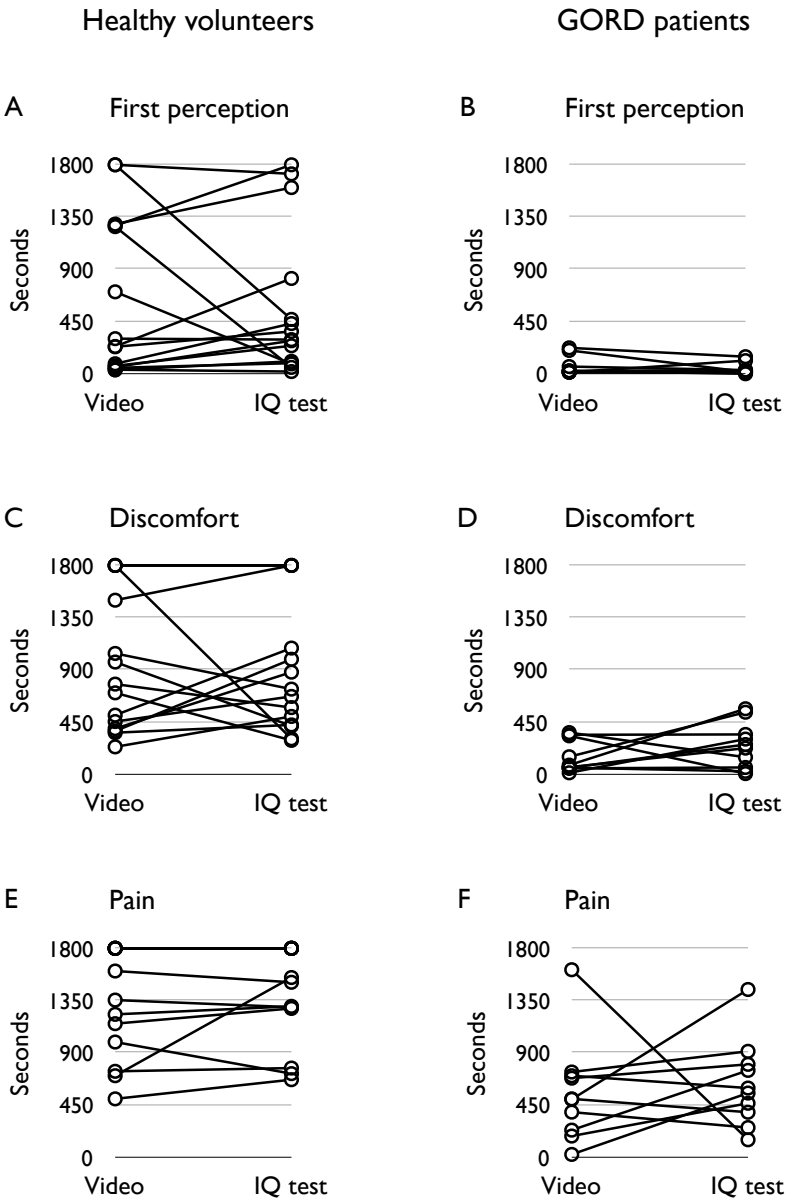

Figure 2 Individual results of time (seconds) needed to provoke first perception, discomfort and pain in healthy volunteers (A, C and E, respectively) and GORD patients (B, D and F, respectively) after start of acid infusion in both measurements. After 30 minutes (1800 seconds) the acid infusion was discontinued if pain threshold was not reached.

Differences between healthy volunteers and GORD patients

GORD patients had significantly higher state (STAI-DY1; $p<0.01$) and trait anxiety scores (STAI-DY2; $p<0.01$) and larger increase in diastolic blood pressure ($p<0.05$) during the stress task compared with healthy volunteers.

No differences were found between GORD patients and healthy volunteers in baseline systolic and diastolic blood pressures. Increase of systolic blood pressures during the stress task was larger in GORD patients but the difference did not reach statistical significance. During the acid perfusion test less time was needed to provoke first perception, discomfort and pain in GORD patients than in healthy volunteers (figure 2).

DISCUSSION

In this study acute psychological stress was found not to affect perception of acid infused into the oesophagus, neither in healthy volunteers, nor in patients with documented GORD. Both in the healthy volunteers and in GORD patients the stress task applied resulted in significantly elevated systolic blood pressures indicating that the applied stressor was effective. We chose to use a modified IQ test because an uncontrollable performance task with social-evaluative threat is considered to be the most efficient acute laboratory stressor [10, 13, 14]. This stress task has been validated before and was shown to increase blood pressures [9].

In agreement with findings reported by Smith et al, our GORD patients almost immediately experienced heartburn when the acid was infused in the oesophagus (lag time to first perception <30 seconds in both measurements) [15]. As we included patients with a good symptom association between symptoms and reflux episodes, a short time lag to first perception is not very surprising. With short time lags from start of acid infusion to first perception, differences during a stress task will be small as well, and therefore more difficult to detect with a small sample size. Thus, the small number of GORD patients may have led to a type II error. However, the stress task not only failed to have an effect on time required to provoke initial symptom perception, but also on time necessary to provoke discomfort or pain.

A possible explanation for the lack of an effect of acute stress on oesophageal acid perception is that sustained chronic stress might be more important than acute psychological stress in the development of oesophageal hypersensitivity. It has been shown that the presence of a severe sustained life event is related to increased severity of heartburn symptoms during the following months [1].

Second, our results might be influenced by the effect of distraction during the IQ test. In order to minimize this effect, the subjects were repeatedly asked, at 1-minute intervals, whether their sensation changed, both during the control and the stress task. It is unclear to what extent this repeatedly and consistently prompting of sensation of symptoms has overcome this effect.

Our results are in conflict with the results of a recently published study performed by Fass et al. [7]. During an acid perfusion test in GORD patients, time lag from start of acid infusion to initial symptom perception was reduced from approximately 4 minutes during a control task to approximately 2 minutes when patients were exposed to an auditory stressor. This was found despite the lack of increased hormonal or autonomic parameters of stress response [7, 16]. The most striking difference with our study is the relatively large time lag from the start of acid infusion to first perception in GORD patients. This may be caused by differences in patient selection, as the patients studied by Fass and colleagues were not selected on the basis of symptom association analysis. As a result, their patients may have been less sensitive to acid than ours.

In our study some differences between the healthy volunteers and GORD patients were found. Although state and trait anxiety levels of GORD patients were comparable with data from previous studies, our GORD patients had higher STAI scores for both state and trait anxiety levels than healthy volunteers [17, 18]. Theoretically, GORD patients may have been more stressed before the start of the measurement than healthy volunteers, and as a result, no differences in lag times between control and stress condition were observed in GORD patients. However, state anxiety levels did not differ between both occasions in both healthy volunteers and GORD patients and a clear rise in systolic blood pressure was seen in both groups during the stress condition. Furthermore, no significant relationships were found between STAI-DY scores and differences in time needed to reach the perception levels (first perception, discomfort and pain) of both healthy volunteers and GORD patients.

In the majority of the healthy volunteers, initial perception of heartburn was provoked by acid infusion during both measurements. This is in contrast with another study where only 20% of (mainly Chinese) healthy volunteers experienced heartburn after 20 minutes of acid infusion [19]. In our study, acid infusion had to be discontinued because of pain in 8 and 9 healthy volunteers during the control and stress task, respectively. As expected, we were able to elicit heartburn in all GORD patients during both measurements. None of the GORD patients were able to complete the 30-minute acid infusion period because of painful heartburn.

Compared with the GORD patients, healthy volunteers had significantly longer lag times to first perception, discomfort and pain thresholds, indicating that healthy volunteers are less sensitive to acid infusion than GORD patients.

In conclusion, the stress-inducing task used in this study did not increase oesophageal acid perception, neither in healthy volunteers nor in acid-sensitive GORD patients. The observed increase in systolic blood pressure strongly suggests that the experimental stressors were effective.

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Relationship between Gastro-oesophageal Reflux Pattern and Severity of Mucosal Damage

6

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ABSTRACT

Objective: To compare the characteristics of reflux episodes in controls and in patients with various degrees of oesophagitis and Barrett's oesophagus.

Methods: Ambulatory 24-hour impedance-pH tracings were analyzed from healthy volunteers, patients with non-erosive reflux disease (NERD), patients with grade A oesophagitis, grade B oesophagitis, grade C or D oesophagitis and patients with a short segment (< 2 cm) of Barrett's metaplasia.

Results: The number of acid and weakly acidic reflux episodes increased from 25.9 ± 3.9 and 17.9 ± 1.5 in the controls, 39.9 ± 6.3 and 33.4 ± 5.7 in the patients with NERD, 46.6 ± 6.2 and 40.4 ± 9.2 in LA A, 68.2 ± 9.2 and 49.2 ± 12.3 in LA B, 79.8 ± 15.6 and 47.4 ± 4.6 in LA C/D to 75.1 ± 7.9 and 37.3 ± 8.5 in the patients with Barrett. The proportion of reflux episodes that is acidic or alkaline was similar all groups. Comparison with normal values revealed that none of the controls, 40% of the patients with NERD, 50% of the patients with LA A, 80% of the patients with LA B and all patients with LA C/D or Barrett's oesophagus had an abnormally high total number of reflux episodes. In the patients with severe oesophagitis a significantly higher percentage of reflux episodes reached the proximal oesophagus (43.8%) compared to the patients with Barrett's oesophagus (19.2%).

Conclusions: With increasing degrees of oesophagitis, patients have more reflux episodes but a large overlap between the groups exists making comparison to normal values of limited relevance. In patients with Barrett's oesophagus fewer reflux episodes reach the proximal oesophagus which might explain their low sensitivity to reflux.

INTRODUCTION

The majority of patients who present with symptoms suggestive for gastro-oesophageal reflux disease do not have mucosal damage, as observed during upper endoscopy. Either these patients do not have reflux disease, already use acid suppressive medications that healed previous erosions or simply never have developed oesophagitis. Thus, only a minority of the patients with reflux symptoms presents with erosive oesophagitis or Barrett's oesophagus. Patients with erosive oesophagitis and Barrett's oesophagus have a higher oesophageal acid exposure, compared to patients with non-erosive reflux disease (NERD) and healthy controls [1-5]. While patients with mucosal lesions have a higher oesophageal acid exposure, it is not known whether their reflux patterns differ only in quantity of acid exposure or that also differences exist in composition, proximal extent and volume of the reflux episodes. It has been suggested that patients with more severe reflux disease have relatively more acid reflux compared to healthy subjects, suggesting differences in gastric acid distribution but this could not be confirmed by others [6, 7].

Various publications have shown that the proximal extent of reflux episodes is an important determinant of whether or not a reflux episode is perceived [8, 9]. It has been suggested that a relatively high proportion of proximally extending reflux episodes causes the high sensitivity to reflux in patients with a relatively low acid exposure and that reduction of the proximal extent of reflux episodes subsequently results in a reduction of reflux symptoms [10, 11]. On the other hand it could be that patients with Barrett's oesophagus have a relatively high proportion of short segment reflux episodes explaining why these subjects have metaplasia only in the most distal oesophagus and why they are often relatively insensitive to gastro-oesophageal reflux [12]. While differences in reflux patterns are thus certainly important and potentially have clinical implications, they are not studied well and comparative data between various degrees of reflux disease is lacking. We hypothesize that patients with Barrett's oesophagus have a both quantitatively and qualitatively different reflux profile compared to the other groups, which in part would explain their paradoxically low sensitivity to acid reflux.

The aim of this study was therefore to compare the characteristics of acid, weakly acidic and weakly alkaline reflux episodes in controls and in patients with various degrees of oesophagitis and Barrett's oesophagus.

METHODS

Subjects

Esophageal impedance-pH tracings were obtained from 10 healthy volunteers and from 50 patients with gastro-oesophageal reflux disease (GORD). The healthy volunteers did not have

a history of gastro-intestinal surgery, not did they have gastrointestinal symptoms. The patients studied were 10 consecutive patients with non-erosive reflux disease (NERD), 10 patients with grade A oesophagitis, 10 patients with grade B oesophagitis, 10 patients with grade C or D oesophagitis and 10 patients with a short segment (< 2 cm) of Barrett's metaplasia. Patients with a longer Barrett segment were excluded as it is currently not clear whether the interpretation of impedance tracings is reliable in these subjects. The degree of oesophagitis was classified according to the Los Angeles classification [13]. All patients with NERD were responsive to acid inhibitory drugs earlier, indicating that their symptoms were acid reflux-related.

All patients suffered from typical reflux symptoms (heartburn and/or regurgitation or chest pain. Written informed consent was obtained from all subjects and the protocol was approved by the medical ethical committee of the University Medical Center Utrecht, the Netherlands.

Study protocol

The use of gastric acid-inhibitory drugs and drugs that might influence gastrointestinal motility was discontinued at least 5 days before the measurement. Stationary oesophageal manometry was performed to determine the distance from the nostrils to lower oesophageal sphincter (LOS). Thereafter, the combined impedance-pH catheter was introduced transnasally and positioned based on the manometric findings (see below).

Patients were instructed to consume 3 meals and 4 beverages at fixed times during the 24-hour measurement period and to note these in a diary. The period spent in supine position was also noted in the diary.

Intraluminal impedance and pH monitoring

A combined pH-impedance recording system was used consisting of a pH-impedance catheter which enabled recording from 6 impedance segments, each recording segment being 2 cm long, and one pH sensor and a portable datalogger (Ohmega, MMS, Enschede, the Netherlands). The impedance recording segments were located at 2-4, 4-6, 8-10, 10-12, 14-16 and 16-18 cm and the pH sensor was located at 5 cm above the upper border of the manometrically localized LOS. Signals were stored in a digital system using a sample frequency of 50 Hz for impedance signals and 2 Hz for pH signals. Intraluminal pH monitoring was performed with a antimone pH electrode (Versaflex, Alpine Biomed, Fountain Valley, California, USA).

Data analysis

In the analysis of the impedance tracings, gas reflux was defined as a rapid (>3000 Ω/s) and pronounced retrograde moving increase in impedance in at least two consecutive impedance sites [14]. Liquid reflux was defined as a fall in impedance of ≥ 50 % of baseline impedance that

moved in retrograde direction in the two distal impedance sites. Mixed liquid-gas reflux was defined as gas reflux occurring during or immediately before liquid reflux. Liquid and mixed reflux episodes were classified as acidic when the pH dropped below 4; reflux episodes were classified as weakly acidic when nadir pH was between 7 and 4 [15]. Weakly alkaline reflux was defined as liquid or mixed reflux with a nadir pH above 7. In the analysis, the periods of meal consumption were disregarded.

Reflux episodes were considered to have reached the proximal oesophagus when they had a proximal extent of at least 15 cm above the LOS (most proximal 2 impedance segments). Data was compared with the normal values published by Zerbib et al [16].

The temporal relationship between symptoms and reflux episodes is expressed using the symptom association probability (SAP) [17].

Statistical analysis and presentation of data

Comparisons between normally distributed data were performed using one-way ANOVA followed by least significance difference (LSD) pairwise multiple comparison tests and between not normally distributed data using the Kruskal-Wallis test. Differences were considered statistically significant when $p \leq 0.05$. Throughout the manuscript parametric data are presented as mean ± SEM or medians.

RESULTS

Demographic data of the controls and the patients are listed in table 1. The prevalence of a sliding hiatal hernia was 20 % in the patients with NERD, 50 % in both the patients with grade A oesophagitis and the patients with grade B oesophagitis, 80 % in the patients with grade C or D oesophagitis and 40 % in the patients with a short segment of Barrett's epithelium. Of the patients with NERD, 50% of the patients had a positive SAP for either acid or weakly acidic reflux. The patients with LA grade A, B and C/D oesophagitis had a positive SAP in 60%, 50% and 30%. Forty percent of the patients with Barrett's oesophagus had a positive SAP.

The acid exposure time was not different between the controls and patients with NERD but increased with the more severe degrees of oesophagitis, with the patients with grade C or D oesophagitis or Barrett's oesophagus having the highest acid exposure time, although a large overlap between the different groups exists (figure 1). Also, a higher number of acid and weakly acidic reflux episodes was observed in the patients with increasing degrees of oesophagitis and Barrett's oesophagus (figure 2). The proportion of total reflux episodes that was acidic and alkaline was similar in all groups and varied only little between the controls (58.4 % and 1.3 %), NERDs (53.9 % and 0.9 %), grade A oesophagitis (53.0 % and 1.1 %),

grade B oesophagitis (57.8 % and 0.5 %) and grade C and D oesophagitis (62.3 % and 0.7 %) and Barrett's oesophagus (66.3 % and 0.7 %) (figure 2).

	Age (yrs)	Range (yrs)	% male
Controls	42	23-64	60
NERD	44	25-72	40
Oesophagitis LA A	48	31-58	50
Oesophagitis LA B	52	25-62	50
Oesophagitis LA C/D	45	37-59	60
Barrett's oesophagus	49	33-61	60

Table 1 Demographics of patients and controls

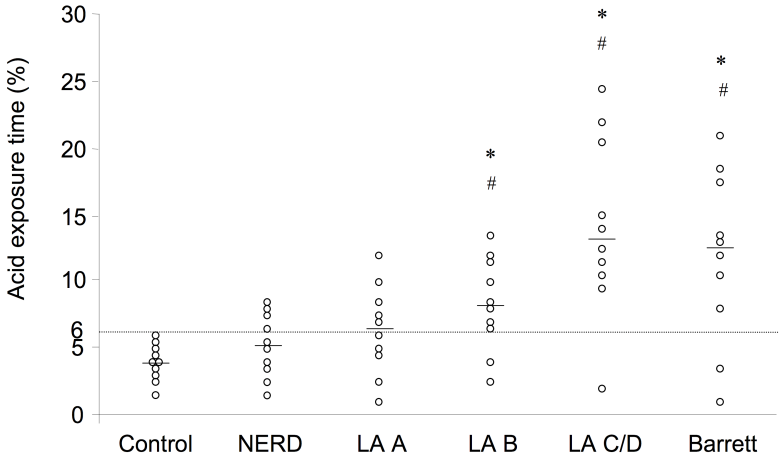

Figure 1 Acid exposure time in controls and in patients with various degrees of reflux oesophagitis. * $p < 0.05$ versus controls; # $p < 0.05$ versus NERD

Both the number of pure liquid and the number of mixed liquid-gas reflux episodes increased with increasing severity of oesophagitis and Barrett's oesophagus (figure 3). The median nadir pH reached during reflux episodes was lower in the patients with oesophagitis (LA A 3.2±0.2, LA B 2.6±0.1, LA C/D 2.8±0.5) and Barrett's oesophagus (2.5±0.2) compared to the controls (3.8±0.3) and the patients with non-erosive reflux disease (3.6±0.4)($p<0.05$). The average drop in pH during reflux episodes did not differ between the different groups.

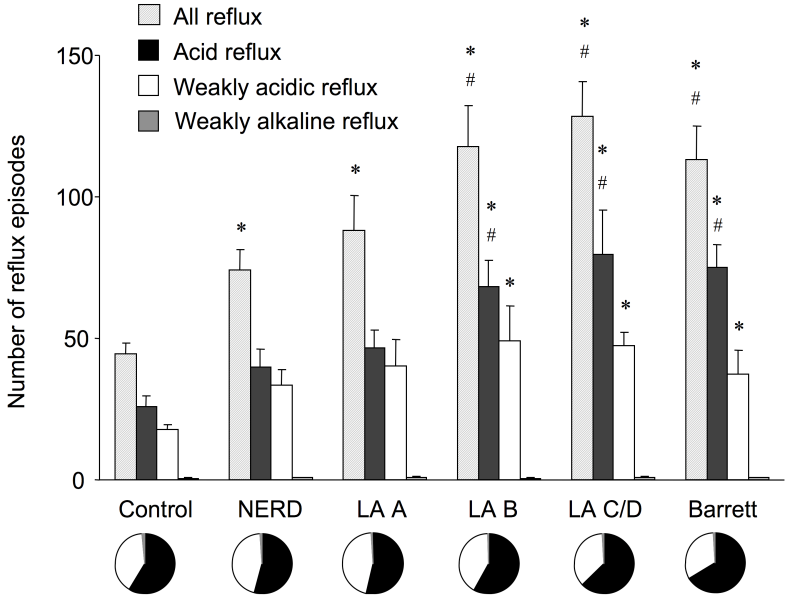

Figure 2 Number of reflux episodes and proportion of total reflux episodes that is acidic, weakly acidic and weakly alkaline in controls and in patients with various degrees of reflux esophagitis. * $p < 0.05$ versus controls; # $p < 0.05$ versus NERD

Patients with severe oesophagitis (grade C/D) and patients with Barrett's oesophagus had a significantly longer acid clearance time compared to the controls and the other patients (figure 4). The bolus clearance time was significantly longer in the patients with severe oesophagitis (grade C/D) compared to the controls and the patients with NERD, while no differences were found between the patients with Barrett's oesophagus and the other groups (figure 5).

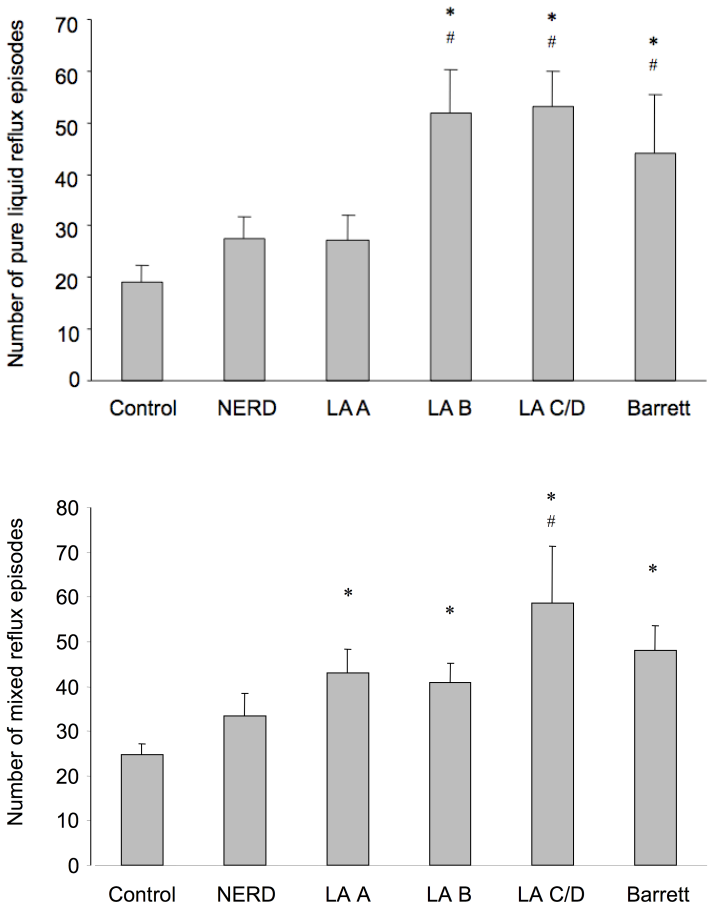

Figure 3 Number of pure liquid and number of mixed liquid-gas reflux episodes in controls and in patients with various degrees of reflux oesophagitis. * $p < 0.05$ versus controls; # $p < 0.05$ versus NERD

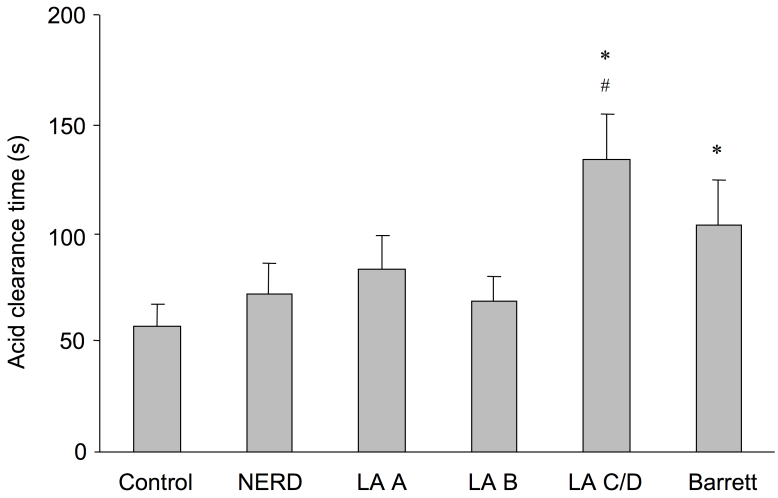

Figure 4 Acid clearance time of reflux episodes in controls and in patients with various degrees of reflux oesophagitis. * $p < 0.05$ versus controls; # $p < 0.05$ versus NERD

According to the normal data of Zerbib et al. none of the controls, 40% of the patients with NERD, 50% of the patients with grade A oesophagitis, 80% of the patients with grade B oesophagitis and all patients with grade C or D oesophagitis or Barrett's oesophagus had an abnormally high total number of reflux episodes. The percentage of subjects with an abnormal number of acid reflux and weakly acidic reflux episodes was respectively 10% and 0% in the controls, 20% and 50% in the patients with NERD, 50% and 50% in the patients with grade A oesophagitis, 80% and 70% in the patients with grade B oesophagitis, 60% and 100% in the patients with grade C/D oesophagitis and 90% and 50% in the patients with Barrett's oesophagus.

In the patients with severe oesophagitis (grade C/D) a significantly higher percentage of reflux episodes reached the proximal oesophagus compared to the patients with Barrett's oesophagus, while no differences were found between the other groups (figure 6).

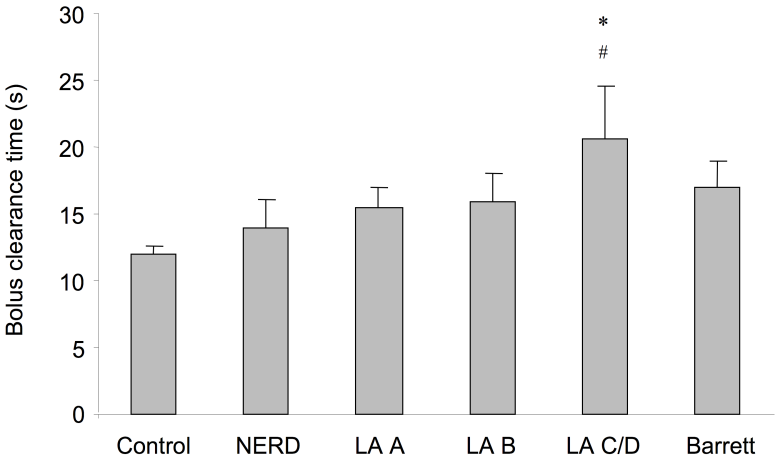

Figure 5 Bolus clearance time of reflux episodes in controls and in patients with various degrees of reflux oesophagitis. * $p < 0.05$ versus controls; # $p < 0.05$ versus NERD

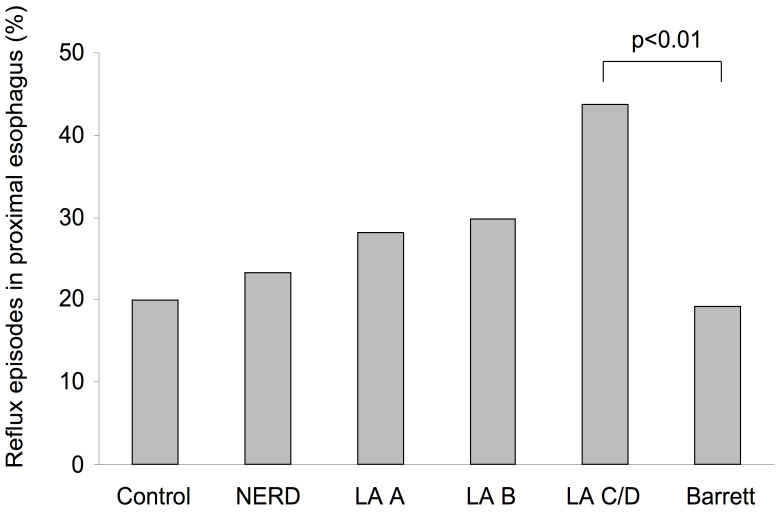

Figure 6 Proportion of reflux episodes reaching the proximal esophagus in controls and in patients with various degrees of reflux oesophagitis.

DISCUSSION

This study confirms earlier findings that with increasing severity of mucosal damage there is an increase in oesophageal acid exposure. A novel finding is that not only the number of acid reflux episodes but also the number of weakly acidic reflux episodes is increased in patients with severe oesophagitis and Barrett's oesophagus. The failure of the anti-reflux barrier that causes GORD is not selective for either acidic or weakly reflux, as both reflux episodes are increased in the patients with GORD and the proportion between the two types of reflux episodes is similar in all groups. A gradual increase in pure liquid and mixed liquid-gas reflux episodes was found in the patients with increasing degrees of oesophagitis. The acid exposure time of the patients with short-segment Barrett's oesophagus is comparable to the patients with more severe oesophagitis. It is important to realize however, that the overlap in acid exposure time and number of reflux episodes between the different groups is large, and that acid exposure time and number of reflux episodes are related to mucosal damage but do not predict this reliably. The sometimes observed discrepancy between severity of acid exposure and mucosal damage can be explained by the fact that mucosal damage is not only dependent on number and pH of reflux episodes but also on the mucosal resistance and the presence of bile acids and pepsin in the refluxate [18]. Besides an increasing number of reflux episodes also the duration of reflux episodes and the proximal extent of the reflux episodes was larger in the patients with increasing degrees of oesophagitis. Longer acid and bolus clearance times suggest higher volumes of reflux as well as an impaired clearance of refluxate [19].

As acid exposure is related but does not predict mucosal damage, it can not be used to diagnose GORD as for the diagnosis of GORD either mucosal damage of symptoms related to reflux need to be present. A high acid exposure does not predict oesophagitis, neither does it prove that symptoms are related to reflux. On the other hand, a very high acid exposure time was only found in those with oesophagitis and Barrett. Comparison with normal values revealed that between 40 and 100 % of the patients had an abnormally high number of total reflux episodes, while between 20 and 90% of the patients had an abnormally high number of acid reflux episodes and between 50 and 100% of the patients had an abnormally high number of weakly acidic reflux episodes. This implies that, when using a certain number of reflux episodes as a cut-off to diagnose GORD, many patients will be missed and a low sensitivity will result. We therefore argue that comparing the number of reflux episodes, either acid or weakly acidic, with normal values is of limited value in the clinical workup of a patient with reflux symptoms.

The number and duration of acidic and weakly acidic reflux episodes increased with increasing severity of oesophagitis and the reflux pattern found in the patients with Barrett's oesophagus differed only in proximal extent from the patterns found in the other groups.

Earlier studies have shown that reflux episodes reaching the proximal oesophagus are important in triggering symptoms, and perhaps the limited number of proximal reflux episodes in the patients with Barrett's oesophagus can, in part, explain why patients with Barrett's oesophagus often report relatively few symptoms [8]. Furthermore, the high acid exposure just above the squamocolumnar junction can explain why the patients all have only a short segment of intestinal metaplasia. While in most studies patients with Barrett's oesophagus have a higher oesophageal acid exposure compared to patients with severe oesophagitis, this was not the case in our study [18, 20]. This can also be explained by the fact that we only included patients with a short segment Barrett's epithelium, while the degree of oesophageal acid exposure is related to the length of the Barrett segment, and in other studies patients usually had longer Barrett segments [4]. Recently, it has been shown that experimentally-induced oesophagitis leads to more proximally extending reflux episodes [21]. In our study, a trend towards an increased proximal extent of reflux episodes was observed with increasing severity of oesophagitis but this did not reach significance. Furthermore, the proximal extent of patients with NERD was not significantly higher than the extent of reflux episodes of the healthy controls, which is apparently in contrast to an earlier study of our group [9]. However, in that study the patients with an increased proximal extent were all SAP positive, while this is not the case in the patients with NERD in the current study.

Although it has often been suggested that duodenogastro-oesophageal reflux plays an important role in the pathogenesis of Barrett's oesophagus, patients with Barrett oesophagus did not have a higher proportion of weakly alkaline reflux episodes. At first, this seems paradoxical, however weakly alkaline reflux is not synonymous to duodenogastro-oesophageal or bile reflux. In a normal anatomic situation bile has to pass the stomach before it can enter the oesophagus. A relatively low volume of bile is mixed intra-gastrically with a large volume of acidic juice before it can enter the oesophagus. Therefore, bile reflux is probably most often acidic. The finding of a low number of alkaline reflux episodes thus does not exclude bile as a causative agent in the pathogenesis of Barrett metaplasia.

In summary, this study shows that with increasing degrees of oesophagitis patients have more and longer acid and weakly acidic reflux episodes, while weakly alkaline reflux episodes are rare in all groups. While the mean values of acid and weakly acidic reflux episodes are very different between the different patients groups and are related to severity of mucosal damage, a large overlap exists. We conclude that the large overlap in isolated parameters such as number of reflux episodes or acid exposure between the various groups implies that comparison to normal values is only of limited relevance and can not be used to diagnose reflux disease. In patients with Barrett's oesophagus only little reflux episodes reach the proximal oesophagus which can explain why these subjects have a low sensitivity for gastro-oesophageal reflux.

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Increased swallowing frequency in GORD is likely to be caused by perception of reflux episodes

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ABSTRACT

Background & Aim: Patients with gastro-oesophageal reflux disease (GORD) swallow air more frequently and have more gas-containing reflux episodes than healthy controls. One explanation for this phenomenon may be that GORD patients primarily swallow more frequently and, as a consequence, have more swallow- or transient lower oesophageal sphincter relaxation-associated reflux episodes. Another explanation may be that GORD patients swallow more often in response to perception of reflux episodes. The aim of this study was to differentiate between these two possible mechanisms.

Methods: In 34 patients with typical reflux symptoms oesophageal 24-h pH-impedance monitoring was performed twice, once off and once on PPI therapy. The number of reflux episodes and number of swallows and air swallows was evaluated. The symptom association probability (SAP) was used to distinguish patients with a good relationship between symptoms and reflux episodes (SAP+) from those who had not (SAP-).

Results: In both the SAP+ (n=21) as SAP- patients (n=13) the acid exposure time decreased during PPI therapy. In the SAP+ patients the number of swallows decreased on PPI (829 ± 85 off vs. 701 ± 79 on PPI, $p<0.05$), whereas in the SAP- patients, the incidence of swallows (802 ± 93 off vs. 814 ± 69 on PPI, p=NS) was not influenced by the PPI therapy.

Conclusion: PPI therapy reduces the number of swallows in patients with a positive SAP, but not in those with a negative SAP. This finding supports the hypothesis that the increased incidence of swallows in GORD is brought about by responses to perceived reflux events.

INTRODUCTION

Swallowing, or deglutition, is the mechanism that results in transport of oral contents from the buccal cavity through the pharynx and the oesophagus into the stomach. The swallowing process is a complex motor pattern in which several functional cortical sensorimotor regions as well as brainstem and cerebellar regions are involved [1-3]. The oral phase of the swallowing process is voluntary in contrast to the pharyngeal and oesophageal phase.

Frequently, a small amount of air accompanies the swallowed bolus or saliva [4]. The ingested air accumulates in the proximal stomach and can be vented during transient lower oesophageal sphincter relaxations (TLOSRs). TLOSRs are the result of a vagally mediated reflex that can be provoked by gastric distension and act as a physiological protection mechanism that prevents extreme gastric dilatation by gaseous contents [5-9]. The vast majority of gastro-oesophageal reflux events occurs during either TLOSRs or swallow-associated LOS relaxations [10-13].

With the use of intraluminal impedance monitoring, swallow-induced aboral bolus transit of air and fluids can be accurately identified and swallows with and without air preceding the liquid bolus can be distinguished [14].

Bredenoord et al. showed that in healthy controls the rate of air swallows is correlated to both the intragastric air volume and the number of gaseous reflux episodes [15]. In patients with gastro-oesophageal reflux disease (GORD) air swallows and gas-containing reflux episodes were found to occur more frequently than in healthy controls [16].

At present it is unknown why GORD patients exhibit more (air) swallows than normal controls. Since the swallowing process is initiated voluntarily, it can be hypothesized that GORD patients primarily swallow more frequently, leading to a secondary increase in swallow- or TLOSR-associated reflux episodes. An alternative explanation for the high swallowing frequency is that the increased swallowing frequency in GORD patients is triggered by the perception of reflux episodes.

To differentiate between these two possible mechanisms we investigated whether the number of swallows is affected by PPI therapy, since PPIs decrease the acid exposure time and the number of perceived reflux events [17-20] and do not affect the number of reflux episodes [21, 22].

METHODS

Subjects

The patients included in this sbtudy were recruited from those attending the out-patient gastroenterology clinics of the St Antonius Hospital, Nieuwegein and the University Medical Center, Utrecht, the Netherlands. Patients presented with typical reflux symptoms such as

heartburn, chest pain and/or regurgitation [23]. Patients with a history of surgery of the stomach or oesophagus were excluded.

Study Protocol

All patients underwent ambulatory 24-h pH impedance monitoring twice. One measurement was performed after cessation of PPI therapy for 7 days, and one during double-dose PPI therapy. Patients used different PPIs, but double-dose therapy was prescribed in all of them. The patients were explicitly instructed to take the PPI twice a day, half an hour before breakfast and evening diner. There was a time interval of at least one week between both measurements and the order of the measurements was randomized. In addition, all patients underwent an upper endoscopy during PPI therapy. The protocol was approved by the medical ethical committees of the hospitals involved. All patients gave written informed consent.

Impedance and pH monitoring

Prior to the ambulatory pH-impedance measurement, all subjects underwent a stationary oesophageal manometry in order to locate the upper border of the lower oesophageal sphincter (LOS).

A combined pH-impedance catheter (VersaFlex, Alpine Biomed, Fountain Valley, California, USA) was used for the ambulatory measurements. This catheter has a single antimony pH electrode and 8 ring electrodes enabling impedance recording from 6 segments. The pH-impedance catheter was positioned with the pH electrode 5 cm above the upper border of the LOS and the impedance recording segments located at 2-4 cm, 4-6 cm, 6-8 cm, 8-10 cm, 14-16 cm and 16-18 cm above the upper margin of the LOS.

All signals were stored in a digital datalogger (Ohmega, MMS, Enschede, The Netherlands) using a sample frequency of 50 Hz for the impedance and 1 Hz for the pH signals.

During the measurement all patients were instructed to note all eating and drinking periods and time spent in recumbent position in a diary. In addition, all patients were instructed to press the event marker button on the datalogger whenever they experienced a symptom and to describe the nature of this symptom in the diary.

Data analysis

The analysis of the pH and impedance recordings was performed manually after completion of the last measurement.

Reflux events were detected in the impedance tracings and were classified according to their nadir pH into acid (nadir pH below 4), weakly acidic (pH between 4 and 7) and weakly alkaline reflux (pH above 7) [24]. Reflux events were defined as >50% impedance drop from baseline starting in the most distal recording segment and moving in retrograde direction.

In addition, the numbers of swallows with and without air component were evaluated. Swallows were defined as a decrease in impedance moving from the most proximal to the most distal recording segment. An air-containing swallow was identified when the aborally propagated impedance fall was immediately preceded by an increase in impedance of at least 1000 Ω in the most distal recording segment [14].

Distal oesophageal acid exposure time, defined as the percentage of time with a pH below 4, was evaluated for both measurements.

Symptom association analysis was performed to assess the relationship between symptoms and reflux episodes. All types of reflux episodes (acid, weakly acidic and weakly alkaline reflux episodes) were taken into account. When a symptom occurred in the 2-minute time window after the start of a reflux event, the reflux episode was considered to be symptomatic [25]. The symptom association probability (SAP), calculated according to Weusten et al. [26], was used to describe the likelihood that symptoms are related to reflux episodes. When the SAP was ≥95% the symptoms were considered to be related to gastro-oesophageal reflux. A negative SAP (SAP<95%) indicates a lack of association between symptoms and reflux episodes. In patients with a negative SAP it is likely that their symptoms are derived from another cause.

Patients who had a positive SAP (SAP≥95%) in one or both measurements were considered to be reflux-sensitive and were distinguished from those who had a negative SAP (SAP<95%) in both measurements.

Statistical Analysis

Normally distributed data is expressed as mean ± SEM. When data is not normally distributed the data is expressed as median and interquartile ranges.

The paired Student T test or the Wilcoxon signed rank test was used to compare both measurements in the same patients. To compare the SAP-positive and the SAP-negative patients an independent T-test or the nonparametric Mann-Whitney U test was performed, the latter when data was not normally distributed.

To investigate the relationship between swallows and air swallows and number of reflux events Pearson's correlation coefficient was calculated. Spearman's correlation calculation was used to assess the relationship between number of swallows and air swallows and the acid exposure time.

RESULTS

Patients

Forty-one patients were included in the study. Seven patients were excluded, 3 because of failure of the hardware and 4 because they were not willing to undergo the second measurement. Thirty-four patients successfully underwent both ambulatory pH-impedance measurements off and on PPI.

Upper endoscopy revealed erosive disease in 5 patients only. According to the Los Angeles criteria, 1 patient had grade A oesophagitis, 2 patients had grade B oesophagitis and 2 patients had grade C oesophagitis.

Symptom association analysis

Of the 34 patients, 21 had a positive SAP in one or both measurements. The remaining 13 patients were asymptomatic during the 24-hour recording or did not have a good relationship between symptoms and reflux episodes in both measurements.

All patients with erosive reflux disease had a positive SAP.

During both measurements patients with a positive SAP had significantly more symptoms (off PPI: $p<0.01$; on PPI: $p<0.05$) and symptoms related to reflux episodes (off PPI: $p<0.01$; on PPI: $p<0.05$) than patients with a negative SAP (table 1).

The number of symptom episodes was not affected by PPI treatment in the patients with a negative SAP but decreased in the patients with a positive SAP. Likewise, PPI treatment did not affect the number of symptom episodes related to reflux episodes in the patients with a negative SAP but decreased reflux-related symptom episodes in the patients with a positive SAP ($p<0.05$).

		Off PPI	On PPI	p value
SAP<95%	Sx	2 (2 – 3)	1 (0 – 4)	NS
	Sx + Reflux	1 (1 – 1)	0 (0 – 1)	NS
SAP≥95%	Sx	7 (4 – 11)	4 (2 – 7)	<0.05
	Sx + Reflux	4 (3 – 9)	3 (0 – 6)	<0.05

Table 1 Number of symptom episodes and symptom episodes preceded by gastro-oesophageal reflux in patients with a negative SAP (SAP<95%) and in patients with a positive SAP (SAP≥95%), off and on PPI therapy (median, interquartile range). SAP: symptom association probability; Sx: symptom episode

Reflux parameters and swallowing frequency

After cessation of PPI the patients with a positive SAP had significantly larger acid exposure time compared to patients with a negative SAP ($p<0.05$). During PPI therapy no difference in acid exposure time was found between patients with a negative SAP and positive SAP (p=NS). On PPI therapy, acid exposure time decreased in patients with a positive SAP from 7.9% (3.1 – 16.2%) to 1.2% (0.2 – 7.3%) ($p<0.05$) and in patients with a negative SAP from 2.2% (1.2 – 5.2%) to 0.4% (0.2 – 1.4%) (p=NS).

Off PPI therapy, the total number of reflux episodes was higher in the patients with a positive SAP (80 ± 7) compared to patients with a negative SAP (59 ± 7), although differences did not reach the limits of statistical significance (p=0.06). Off PPI therapy, patients with a positive SAP had more acid reflux episodes compared to patients with a negative SAP (SAP-positive: 57 ± 7; SAP-negative: 32 ± 7; $p<0.05$). The number of weakly acid reflux episodes (SAP-positive: 21 ± 3; SAP-negative: 26 ± 4) and weakly alkaline reflux episodes (SAP-positive: 1 ± 1; SAP-negative: 1 ± 1) did not differ between SAP-positive and SAP-negative patients off PPI therapy (p=NS).

On PPI therapy, the total number (SAP-positive: 70 ± 6; SAP-negative: 62 ± 11) and numbers of acid (SAP-positive: 23 ± 5; SAP-negative: 12 ± 6), weakly acidic (SAP-positive: 45 ± 7; SAP-negative: 48 ± 7) and weakly alkaline reflux episodes (SAP-positive: 2 ± 1; SAP-negative: 2 ± 1) did not differ between both groups (p=NS). As expected, during PPI therapy a reduced number of acid reflux episodes and increased number of weakly acidic reflux episodes were identified in both groups.

In the patients with a negative SAP, the incidences of swallows and air swallows were not influenced by PPI therapy. In the patients with a positive SAP the number of swallows decreased under PPI therapy ($p<0.05$), whereas the number of air swallows was not significantly affected (figure 1).

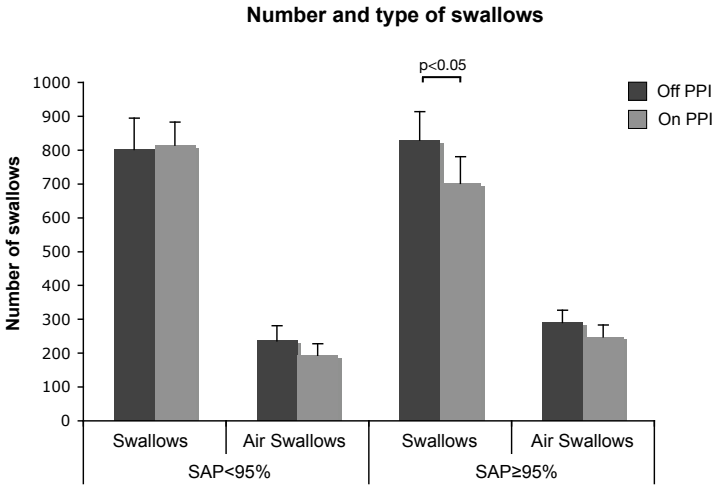

Figure 1 Total number of swallows and air swallows in patients with a negative SAP (SAP<95%) and in patients with a positive SAP (SAP≥95%), both off and on PPI therapy. SAP: symptom association probability

The number of swallows did not differ between patients with a negative and with a positive SAP on both measurements, off and on PPI. In addition, during both measurements, no differences in number of air swallows were found between patients with a negative and with a positive SAP.

In the patients with a negative and in patients with a positive SAP the correlation of number of reflux episodes with swallows or air swallows was not statistically significant, both on and off PPI therapy.

Furthermore, during both measurements on and off PPI, neither in patients with a negative SAP nor in those with a positive SAP significant relationships were found between the acid exposure time and the frequency of swallows or air swallows.

DISCUSSION

From previously published studies it is known that GORD patients swallow air more frequently and have more mixed liquid-gas and pure gas reflux episodes compared to healthy controls [16]. In this study, we aimed to clarify the mechanism that underlies this phenomenon by distinguishing patients who demonstrably perceive reflux episodes from those who do not have a good relationship between symptoms and reflux episodes, and by assessing the effect of PPIs on these parameters.

Our hypothesis was that if GORD patients primarily swallow more frequently, the incidence of swallows would not be influenced by PPI therapy, neither in patients with a negative nor in patients with a positive SAP. On the other hand, if GORD patients swallow in response to perceived reflux events, patients who readily sense reflux episodes (positive SAP) would swallow less frequently during PPI therapy since PPIs reduce the number of symptom episodes and oesophageal acid exposure. In that case, in patients with a negative SAP no differences in swallowing rates were expected during PPI therapy.

We showed that the swallowing frequency only decreases during PPI therapy in patients with a positive SAP and not in those with a negative SAP. This finding suggests that swallowing frequency is affected by the perception of reflux events. The finding that the number of symptom episodes was only reduced with PPI therapy in the SAP+ patients supports this. Patients with a positive SAP tended to have more swallows and air swallows off PPI therapy compared to patients with a negative SAP, although our numbers did not reach the limits of statistical significance. The swallowing frequencies we found in this study are comparable with previously published data from GORD patients and healthy controls [16].

Some weaknesses of out study need to be acknowledged. It is known that oesophageal sensory function differs between patients with erosive and non-erosive reflux disease [27]. Unfortunately, only 5 of the 34 patients had erosive reflux disease. Due to this limited number, comparisons between non-erosive and erosive reflux patients were impeded. Secondly, our patients were not blinded with regard to treatment (on or off PPI), which, theoretically, might have affected the results of the study to some extent.

Swallow-induced oesophageal peristalsis plays an important role in oesophageal emptying and neutralisation of intraluminal acid and thus in the prevention of mucosal damage [28]. Furthermore, the secretion of saliva is enhanced after infusion of acid in the oesophagus in healthy volunteers and patients with reflux symptoms [29-31]. Therefore, the exposure of the oesophageal mucosa to acid may influence the swallowing frequency. However, no arguments for a relationship between oesophageal acid exposure and swallowing frequency were found in our study. Our results indicate that the perception of reflux events affects the swallowing process, rather than the oesophageal acid exposure itself.

In conclusion, PPI therapy reduces the number of swallows in patients with a positive relationship between symptoms and reflux episodes, but not in those without such a relationship. This decrease appears to be independent of the effect of PPI on the occurrence of acidic reflux. These findings support the hypothesis that the increased incidence of swallows in GORD is brought about by responses to perceived reflux events, rather than being the cause of increased gastro-oesophageal reflux.

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Supragastric belching in patients with reflux symptoms

8

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ABSTRACT

Background & Aim: Supragastric belching is a distinct belch pattern found in patients with excessive belching ('aerophagia'). Patients with gastroesophageal reflux disease (GERD) may also complain of belching. It has been shown that GERD patients swallow air more frequently and have more air-containing reflux episodes than healthy controls. It is not known whether supragastric belches occur in patients with reflux symptoms.

Methods: Fifty consecutive patients with typical reflux symptoms and 10 healthy volunteers underwent ambulatory 24-h pH-impedance monitoring off PPI therapy. Patients filled in a questionnaire regarding their symptoms. The number and type of reflux episodes and supragastric belches during the measurement were assessed.

Results: In 24 of the 50 patients with reflux symptoms, supragastric belches were identified with a median incidence of 13 per 24 hour (interquartile range: 6-52). In 5 of the 10 healthy volunteers 2 (1-6) supragastric belches were identified.
In patients with reflux symptoms, 48% of the supragastric belches occurred in close temporal association with reflux episodes. Two different association patterns were observed: in 19 patients supragastric belches occurred immediately prior (<1 second) to the onset of the reflux episode. This pattern was observed in 30% of all supragastric belches. In 15 patients the supragastric belch occurred during the reflux episode, with the onset 4-10 seconds after the start of the reflux episode. This pattern was observed in 18% of all identified supragastric belches.

Conclusions: Supragastric belches occur more frequently in patients with typical reflux symptoms than in healthy subjects. These belches often occur in close association with acid and weakly acidic reflux episodes. Our findings suggest that supragastric belching elicits reflux in some cases and is the patient's response to an unpleasant esophageal sensation in others.

INTRODUCTION

Fifty to 70% of patients with typical reflux symptoms also report belching as a troublesome symptom [1, 2]. In most people, belching is a physiological mechanism that serves to vent ingested air from the stomach. During each swallow, a small amount of air is ingested [3]. Accumulation of air causes distention of the proximal stomach, which elicits a vagally mediated reflex, that results in a transient relaxation of the lower esophageal sphincter (TLESR) [4-7]. During these TLESRs, the trapped intragastric air is vented from the stomach.
Patients whose main symptom is excessive belching are often diagnosed with 'aerophagia' [8]. With the arrival of the impedance monitoring technique new insights have been obtained. We know now that these patients do not swallow air too often or in too large quantities. Neither do they excessively vent gastric air. Instead, a distinct belch pattern can be found, known as supragastric belching. This belch pattern is characterized by a rapid influx of air, observed as an antegrade rise in impedance, immediately followed by a retrograde expulsion of the air seen as a retrograde return to baseline. Air swallowing is not the cause of excessive belching in these patients, but the belches originate from esophageal air ingestion followed by immediate expulsion. A supragastric belch can be discriminated from an air swallow as the influx of air during a supragastric belch is completed within one single second. Moreover, during simultaneous manometry, the air pattern is not accompanied by esophageal peristaltic contractions or by a relaxation of the lower esophageal sphincter [9].
Thus far, it is not known whether patients with reflux symptoms also exhibit supragastric belches. The aim of our study was to describe belch patterns in patients with reflux symptoms.

METHODS

Patients and study protocol

Fifty consecutive patients with typical reflux symptoms, such as heartburn, chest pain and regurgitation were included in this study. Patients filled in a standard questionnaire regarding their (predominant) symptoms and whether they were bothered by frequent belching.
In addition, 10 healthy volunteers without GERD symptoms or frequent belching, were included. The medical ethical committee approved the protocol and informed consent was signed by each subject.
All participants underwent ambulatory 24h pH-impedance monitoring after cessation of acid secretory inhibiting therapy. Proton pump inhibitors (PPIs) and H2-antagonists were discontinued 7 and 3 days prior to the ambulatory study, respectively.

Esophageal impedance and pH monitoring

Stationary manometry was performed in order to locate the upper border of the lower esophageal sphincter (LES). A combined pH-impedance catheter (Versaflex, Alpine Biomed, Fountain Valley, California, USA) was used for the ambulatory measurement and was placed with the antimony pH electrode located at 5 cm above the upper border of the LES. This catheter has 8 ring electrodes that enable impedance recording from 6 segments (2-4 cm, 4-6 cm, 6-8 cm, 8-10 cm, 14-16 cm and 16-18 cm above the upper margin of the LES).

The impedance and pH signals were stored in a digital datalogger (Ohmega, MMS, Enschede, The Netherlands) using a sample frequency of 50 and 1 Hz, respectively.

During the measurement the patients were instructed to consume 3 meals and 4 beverages at fixed times. They were instructed to keep a diary in which they had to note these meal periods and periods that they were in recumbent position. The patients were instructed to press the event marker button on the datalogger whenever they experienced a symptom and to note the time of onset and to describe the nature of the symptom in the diary.

Data analysis

All 24h ph-impedance tracings were analyzed manually. Reflux events were identified on impedance tracings and classified as liquid, mixed liquid-gas and gas reflux, according to previously described criteria [10]. Liquid reflux episodes were defined as an impedance decrease ≥50% from baseline moving in retrograde direction. Gas reflux episodes were defined as a rapid retrograde rise in impedance ≥3000 Ω in at least two consecutive channels. Mixed reflux episodes were defined as liquid reflux episodes accompanied by a gaseous component.

After the identification of the reflux episodes on the impedance tracings, all reflux episodes were classified according to their pH into acid, weakly acidic and weakly alkaline reflux.

Gas-containing reflux episodes (pure gas and mixed liquid-gas reflux episodes) reaching the most proximal impedance recording segment were regarded as gastric belches [11].

The numbers of swallows and swallows accompanied by a gaseous component (air swallows) were assessed. Swallows were defined by a fall in impedance from the most proximal to the most distal recording segment. Air swallows were described as swallows with an impedance rise ≥1000 Ω in the most distal recording segment [12].

Supragastric belches were identified using the criteria described by Bredenoord et al [9]. A supragastric belch was defined as a rapid impedance rise (≥1000 Ω) moving in aboral direction, followed by a return to baseline moving in the opposite direction. This pattern represents rapid esophageal air ingestion immediately followed by air expulsion. Supragastric belches were considered to be related to reflux when a supragastric belch occurred immediately prior (<1 second) to the onset of the reflux episode, or when the supragastric belch occurred during the reflux episode.

Symptom association analysis was performed to assess a relationship between symptoms and reflux episodes. When a symptom occurred in a 2-minute time window following the start of a reflux event, it was considered related to reflux [13]. We used the symptom association probability (SAP) and the symptom index (SI) to describe the statistical probability that symptoms and reflux events were related [14, 15].

Statistical Analysis

Throughout the manuscript the data are presented as mean (± SEM) or as median (and interquartile range). Comparisons between patient groups were performed using the independent t-test or Mann-Whitney U test when parameters followed normal or nonparametric distributions, respectively. The characteristics of the reflux episodes were compared using the paired Student t-test or Wilcoxon signed ranks test. Chi-square test was used to compare the outcome of symptom association analysis between patients with and those without supragastric belches. Oneway ANOVA was used to compare reflux characteristics between healthy volunteers and patients.

To investigate the temporal relationship between supragastric belches occurring immediately prior to the onset of a reflux episode and reflux episodes, the recording period was divided into 1-second time windows. For each second the presence of a supragastric belch, reflux episode or both was evaluated. Hereafter, contingency tables were drawn for every patient who exhibited these types of reflux-related supragastric belches and Fisher's exact test was applied to calculate a p-value.

To investigate the temporal relationship between reflux episodes and supragastric belches occurring during reflux episodes, the same calculation was performed using a 10-second time window. For every time frame, the presence of a supragastric belch and the presence of a reflux episode were evaluated. P-values <0.05 were considered to be statistically significant.

RESULTS

Healthy volunteers

In 5 out of the 10 healthy volunteers 17 supragastric belches were identified. These occurred at median frequency of 2 per 24 hour (IQR: 1-6). All supragastric belches occurred in upright position.

Patients with reflux symptoms

In 24 out of the 50 patients (48%) supragastric belches occurred with a median frequency of 13 per 24 hour (interquartile range: 6-52). In the other 26 patients no supragastric belches were found. Almost all supragastric belches occurred in upright position (100 (98-100)%).

Twenty-five out of the 50 patients reported belching as a troublesome symptom, in addition to their typical reflux symptoms. Supragastric belches were identified in 15 of the 25 patients with frequent belching and in 9 of the 25 patients without this symptom (table 1).

	SBG-	**SGB+**	
Patients without symptoms of belching	16	9	25
Patients with symptoms of belching	10	15	25
	26	24	50

Table 1 Number of patients with symptoms of frequent belching without (SGB-) and with supragastric belching (SGB+). SGB: supragastric belch

In 21 out of the 24 patients with supragastric belches, a substantial proportion (50 (36-82)%) of supragastric belches occurred in close temporal association with liquid-containing reflux episodes. In the other 3 patients, none of the supragastric belches was reflux-related.

Two types of temporal association between reflux episodes and supragastric belches were observed: supragastric belches immediately preceding the onset of reflux (figure 1A) and supragastric belches shortly following reflux onset (figure 1B). In figure 1C an example of a mixed reflux episode is shown in order to clarify the difference with a supragastric belch.

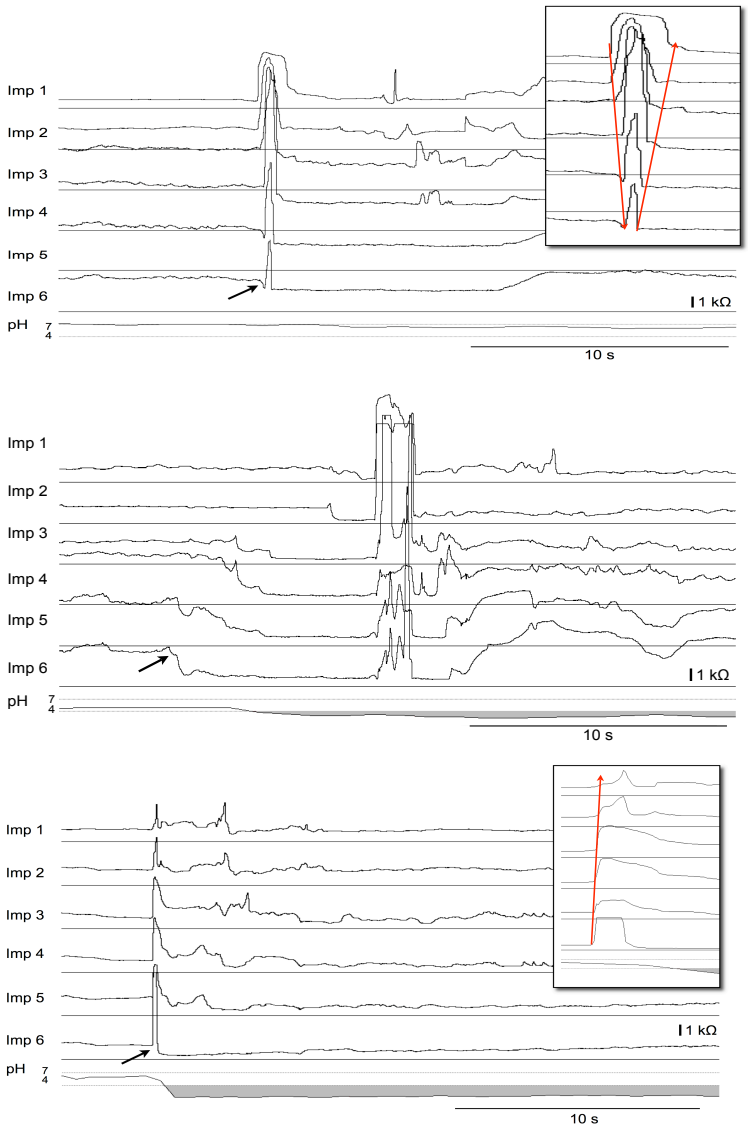

Figure 1 Supragastric belches related to reflux episodes; A) supragastric belch occurring simultaneously with the onset of a weakly acidic reflux episode. The inserted frame shows a magnification of the supragastric belch. B) supragastric belch occurring within seconds after the onset of an acidic reflux episode. C) an example of a mixed liquid-gas reflux episode. The inserted frame shows a magnification of the air-component moving upwards. Black arrows: onset of the reflux episode. Red arrows: direction of air flow.

In 19 patients a statistically significant concentration of supragastric belches in the 1-second window preceding the onset of reflux episodes was observed (Fisher's exact test, $p<0.05$). In these patients, reflux-preceding supragastric belches constituted 33 (20-50)% of the total number of supragastric belches and 75 (39-100)% of the reflux-related supragastric belches.
In 15 patients a statistically significant proportion of supragastric belches occurred within 10 seconds after the onset of a reflux episode ($p<0.01$). This pattern was seen in 20 (14-56)% of the total number of supragastric belches and 52 (25-71)% of all reflux-related supragastric belches in these patients. The median time lag from the onset of the reflux episode to onset of the supragastric belch was 6 (4-10) seconds.
In 13 of the 21 patients, both types of temporal relationships between supragastric belches and reflux events were observed.
In the 24 patients with supragastric belches a total number of 726 supragastric belches were identified of which 48% was related to reflux episodes. Thirty percent of all identified supragastric belches occurred immediately prior to the onset of the reflux episode and 18% followed the reflux episode (figure 2).

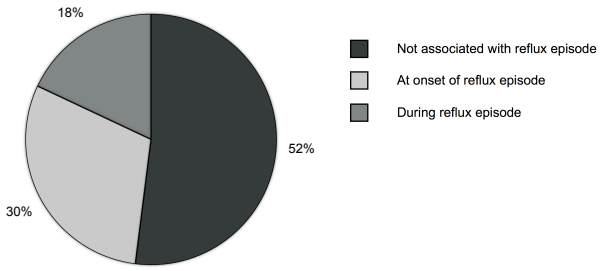

Figure 2 Distribution of the three types of supragastric belches

Reflux episodes associated with supragastric belches did not differ from reflux episodes without supragastric belches with regard to proximal extent (7 (7-9) cm vs. 7 (7-9) cm, respectively, p=NS), volume clearance time (9 (6-12) seconds vs. 9 (6-15) seconds, p=NS), acid clearance time (31 (18-57) seconds vs. 43 (15-64) seconds, p=NS), pH nadir (2.9 (2.2-3.7) vs. 2.6 (2.1-3.2), p=NS), or absolute pH drop (2.5 (2.0-3.5) vs. 3.4 (2.6-4.2), p=NS). In addition, no statistical significant differences between reflux characteristics of reflux events with and without supragastric belches were found in the individual patients.

Of all acidic reflux events, 17 (8-32)% was associated with a supragastric belch and 12 (3-37)% of all weakly acidic reflux events was related to a supragastric belch, the difference not being statistically significant.

Reflux episodes associated with supragastric belches following a reflux episode were symptomatic in 0 (0-33)%, and reflux episodes without supragastric belches were symptomatic in 7 (2-13)%, the difference not being statistically significant. Fifty-four percent of patients with supragastric belches had a positive SAP (≥95%), vs. 42% of the patients without supragastric belches.

Neither the number of reflux episodes or gastric belches, nor the number of swallows or air swallows differed between patients with and patients without supragastric belches (table 2).

	SGB - **N=26**	**SGB +** **N=24**	**p value**
Number of liquid reflux episodes	36 ± 6	31 ± 4	NS
Number of mixed liquid-gas reflux episodes	39 ± 3	41 ± 4	NS
Number of gas reflux episodes	27 ± 4	34 ± 5	NS
Number of gastric belches	63 ± 6	71 ± 7	NS
Number of swallows	818 ± 70	838 ± 72	NS
Number of air swallows	251 ± 28	295 ± 34	NS
Esophageal acid exposure	4.2 (2.0-11.7)%	5.3 (2.6-10.2)%	NS
SAP≥95%	42%	54%	NS
SI≥50%	58%	63%	NS

Table 2 Reflux characteristics, gastric belches, swallows and air swallows per 24 hour, and percentage of patients with positive SAP and a positive SI in patients with supragastric belches (SGB+) and in those without (SGB-)(mean ± SEM). SGB: supragastric belch; SAP: symptom association probability; SI: symptom index

DISCUSSION

This is the first study that describes the occurrence of supragastric belching in a significant subset of patients (48%) with typical reflux symptoms and healthy volunteers (50%).
The incidence of supragastric belches in the patients with reflux symptoms (median 13 per 24 hours) was higher than that observed in healthy subjects (median 2 per 24 hours) but much lower than in patients with 'aerophagia' (excessive belching) [9].
Using impedance monitoring, a supragastric belch can be clearly discriminated from an air swallow. In the latter it takes at least several seconds to travel through the esophagus whereas the influx of air during a supragastric belch is completed within a single second. Manometrically, the absence of an esophageal contraction or relaxation of the lower esophageal sphincter during a supragastric belch helps to distinguish between air swallows and supragastric belches [9].
One could question the clinical relevance of these supragastric belches that occur in patients with reflux symptoms. In our study, almost half of the supragastric belches identified occurred in close association with liquid-containing reflux episodes. In patients and healthy volunteers with reflux-related supragastric belches, we found statistically significant temporal relationships between supragastric belches and reflux episodes, indicating that the above-described patterns are not likely to be caused by mere chance. To calculate the probability that supragastric belches were related to reflux events, we used different time windows for supragastric belches immediately preceding the reflux episodes and supragastric belches occurring after the onset of the reflux episode (1 and 10 seconds, respectively) because we believe these are two distinct phenomena. The characteristics of the reflux episodes (proximal extent, volume and acid clearance time, pH nadir, pH drop) did not differ between reflux episodes without or with the presence of a supragastric belch.
Two patterns of reflux-related supragastric belches were observed. In the first pattern, the supragastric belch preceded the reflux episodes immediately (within 1 second) and in all patients who exhibited this pattern statistical analysis indicated a more than coincidental association between the supragastric belch and the onset of reflux episodes.
Theoretically this association can be explained by the mechanism through which supragastric belching is accomplished. During inspiration with a closed glottis, air is sucked into the esophagus and is immediately expelled by a sudden increase in intrathoracic pressure [9]. The latter probably is the result of abdominal straining as the esophageal pressure and intragastric pressure rise simultaneously. During abdominal straining the increase in intragastric pressure may overcome the pressure at the esophageal high-pressure zone and result in reflux of gastric contents into the esophagus. Another suggestion may be that TLESRs are provoked by supragastric belches through distention of the esophagus. On the other hand, TLESRs may even induce influx of air into the esophagus though a TLESR-associated UES relaxation [16].

The pressure dynamics during these events are very complex, and future research is needed to clarify the underlying mechanism that results in the simultaneous occurrence of reflux episodes and supragastric belches. However, the simultaneous occurrence of the beginning of a reflux episode and a supragastric belch indicates that reflux episodes can be provoked by supragastric belches. Because supragastric belching is a voluntary act, one might hypothesize that in some patients reflux episodes can be reduced by avoiding supragastric belching. A therapeutic approach by means of biofeedback therapy may be of benefit in patients who exhibit this pattern.

The second pattern observed in this study was characterized by a supragastric belch occurring within a few seconds after the onset of a reflux episode. We chose a 10-second time window to assess the temporal relationship between this type of reflux-related supragastric belch and reflux episodes because the median time lag between the onset of the reflux episodes until the occurrence of the supragastric belch was 6 (4-10) seconds. This temporal association suggests that the supragastric belch is the patient's response to an unpleasant esophageal sensation. However, reflux episodes followed by a supragastric belch were not more often symptomatic than reflux episodes without supragastric belches. Nevertheless, it is clear that a voluntary component plays an important role in the genesis of supragastric belches since their frequency is affected by attention and distraction [17].

Belching is a symptom that is reported frequently by patients with reflux symptoms [1, 2]. Fifty percent of our patients with typical reflux symptoms reported belching as troublesome symptom but none reported belching as their predominant symptom. Instead, patients had typical GERD symptoms (heartburn, regurgitation and/or chest pain). This is the first study that describes the presence of supragastric belches in a subset of healthy volunteers and patients with typical reflux symptoms. We showed that supragastric belching is not a specific feature in patients whose main symptom is excessive belching, but occurs in other patients as well. In more than half of all identified supragastric belches, no association with reflux episodes was found. Interestingly, a substantial subset (48%) of supragastric belches occur in close association with gastroesophageal reflux episodes, either immediately before (<1 second) or a few seconds after the onset of a reflux episode. This suggests that supragastric belching elicits reflux episodes in some patients and is a response to an unpleasant esophageal sensation in others.

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Aerophagia: excessive air swallowing demonstrated by esophageal impedance monitoring

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ABSTRACT

Background & aim: Patients with aerophagia suffer from the presence of an excessive amount of intestinal gas, which is thought to result from excessive air ingestion. However, this has not been shown this far. The aim of this study was therefore to assess swallowing and air swallowing frequencies in patients with suspected aerophagia.

Methods: Ambulatory 24-hr pH-impedance monitoring was performed in 10 patients in whom excessive amounts of intestinal gas were visualized on plain abdominal radiograms. All patients had symptoms of bloating, abdominal distention, flatulence or excessive belching. Reflux parameters and number of swallows and air swallows were assessed.

Results: The most common symptoms were bloating, abdominal distention and constipation. Only three patients reported excessive belching and one patient reported flatulence as their predominant symptom. During the 24-hr measurement, patients showed high incidences of air swallows (521 ± 63) and gastric belches (126 ± 37) and normal swallowing frequencies (741 ± 71).

Conclusion: This is the first study that presents objective parameters that confirms the existence of excessive air swallowing or aerophagia using esophageal impedance monitoring.

INTRODUCTION

Bloating, abdominal distention, flatulence, abdominal pain and frequent belching occur frequently in the general population [1]. In the absence of visible abnormalities, these symptoms are often attributed to functional disorders such as functional dyspepsia or irritable bowel syndrome [2, 3].

When patients have excessive amounts of gaseous contents in their gastrointestinal tract, the question arises where this air or gas originates from. Possible explanations may be an increased endogenous production of intestinal gas, provoked by food components such as indigestible carbohydrates or bacterial overgrowth [4], a disturbed gastrointestinal motility that fails to evacuate the accumulated air [5] or excessive (air) swallowing (aerophagia) [6].

The term aerophagia is often used to denote the condition in which patients belch frequently. However, it has been shown that most of these patients do not belch by swallowing air excessively but by sucking it into the esophagus and expelling it immediately thereafter (supragastric belching). In patients with excessive supragastric belching, air is thus not ingested and aerophagia is not a correct term for this disorder.

Excessive air swallowing has anecdotally been incriminated as the cause of acute intestinal complications, such as recurrent ileus, gastric perforation, gastric or colonic volvulus, mainly in case reports on mentally disabled patients [7-10]. These observations suggest that aerophagia can be the cause of symptoms and complications other than belching.

Until recently, it was difficult to study air swallowing reliably for a prolonged period of time. With esophageal impedance monitoring it has become possible to evaluate swallowing frequencies and to discriminate normal swallows from swallows accompanied by air, so-called “air swallows” [11, 12].

The aim of our study was to assess the number of swallows and air swallows in patients in with suspected aerophagia.

METHODS

Patients and study protocol

In this study we included 10 patients with a clinical suspicion of aerophagia, based on indicative symptoms and signs and the presence of excessive amounts of intestinal gas visualized by a plain abdominal radiogram. A large gastric air bubble was seen in 3 patients, in 4 patients excessive amounts of air was seen in the small intestine and in 7 patients colonic dilatation was observed. During physical examination hypertympany was present in 8 patients. Besides the increased amount of intestinal air, no other abnormalities were observed, in particular no air-fluid levels or signs of volvulus (figure 1).

Patients filled in a standard questionnaire regarding their (predominant) symptoms.
All patients underwent ambulatory 24-hr pH-impedance monitoring in order to quantify the (air) swallow frequency and to assess the reflux characteristics.

Ambulatory 24-hr pH-impedance monitoring
A combined pH-impedance catheter (Versaflex, Alpine Biomed, Fountain Valley, California, USA) with 8 ring electrodes enabling impedance recording from 6 segments (2-4 cm, 4-6 cm, 6-8 cm, 8-10 cm, 14-16 cm and 16-18 cm above the manometrically located upper margin of the lower esophageal sphincter (LES)) was used for the ambulatory measurement. The antimony pH electrode was located at 5 cm above the upper border of the LES. The impedance and pH signals were stored in a digital datalogger (Ohmega, MMS, Enschede, The Netherlands) using a sample frequency of 50 and 1 Hz, respectively.
During the measurement the patients were instructed to consume 3 meals and 4 beverages at fixed times. They were instructed to keep a diary in which they had to note these meal periods and the periods during which they were in recumbent position.

Figure 1 Example of a plain abdominal radiogram showing excessive amounts gas in the small intestine and colon

Data analysis

Ambulatory 24-hr pH-impedance tracings were analyzed manually. Periods of meal consumption were excluded from the analysis.

Reflux events were detected using the impedance tracings and classified into liquid, mixed liquid-gas and pure gas reflux episodes [13]. Gas-containing reflux episodes (pure gas and mixed liquid-gas reflux episodes) in which the gaseous component reached the most proximal impedance recording segment were regarded as gastric belches [12].

Swallows were defined as a drop in impedance moving from the most proximal recording segment in aboral direction. Air swallows were defined as a swallow with an impedance rise of $\geq$1000 Ω in the most distal recording segment [11].

Swallowing and air swallowing frequencies were compared with normal values (means and 95th percentiles) derived from previously published data (818 (524-1064)/24-hr and 176 (73-313)/24-hr, respectively) [12] using an unpaired Student's T-test.

Supragastric belches were identified using previously described criteria [14]. A supragastric belch was defined as a rapid impedance rise ($\geq$1000 Ω) moving in aboral direction, followed by a return to baseline moving in the opposite direction within one second.

RESULTS

Patients

Ten patients (mean age ± SD: 43 ± 16y; 6 females) were included. The (predominant) symptoms of each individual are presented in table 1. Constipation requiring laxatives was present in 7 patients. Six of these patients reported constipation as their predominant symptom. Three patients reported excessive belching and 1 patient reported flatulence as their predominant symptom. Three patients had a history of surgery because of abdominal pain which was presumed to be caused by a volvulus (n=2) or ileus (n=1). However, organic abnormalities were never found per-operatively.

Ambulatory 24-hr pH-impedance monitoring

All patients underwent ambulatory 24-hr pH-impedance monitoring. Data from one patient could not be analyzed because of hardware failure.

Patients exhibited 741 ± 71 normal swallows and 521 ± 63 air swallows during the 24-hr recording period. In each patient an abnormally high number of air swallows was found, compared to the normal values ($p<0.001$). No statistically significant differences were found between swallowing frequencies of patients and healthy volunteers ($p=0.59$). Normal numbers of liquid (9 ± 3) and mixed liquid-gas (17 ± 6) reflux episodes were observed in all patients. Patients had excessive numbers of gas-containing reflux episodes (113 ± 37) and gastric belches (126 ± 37).

Only one patient exhibited a small number of supragastric belches (n=3) during the measurement, in the others supragastric belches were not found.

DISCUSSION

This is the first study that provides objective evidence for the existence of the phenomenon known as aerophagia, defined as excessive air swallowing. Our patients had large amounts of intestinal air on plain abdominal radiograms and exhibited impressive numbers of air swallows during ambulatory 24-hr pH-impedance monitoring. In these patients the observed air swallow frequencies (521 ± 63 /24-hr) were much higher than found in healthy volunteers (176 ± 24 / 24-hr) or in GERD patients (291 ± 36 /24-hr) [12, 15].

In addition to an increased air swallowing frequency, large numbers of gas-containing reflux episodes were observed in our patients. It is likely that the increased incidence of gas-containing reflux episodes is caused by gastric distention leading to increased numbers of TLESRs [16-18]. Presumably, of the increased gastric inflow of air part is evacuated through belching and another part is passed through the duodenum.

Our patients reported various symptoms, of which bloating, abdominal distention, constipation and abdominal pain were the most prevalent (table 1). Despite the increased number of gas-containing reflux episodes, only 4 patients reported excessive belching.

These results suggest that aerophagia or excessive air swallowing does not equate to a belching disorder, but is primarily associated with other symptoms. This is in agreement with a previously published study in which patients with aerophagia reported besides excessive belching other symptoms such as abdominal distention, bloating and abdominal pain [19].

Interestingly, seven of ten patients studied reported constipation requiring laxatives. Three patients had undergone surgery because of acute abdominal pain. Despite extensive investigations in these patients (colonoscopy, CT-abdomen, transabdominal echography, ambulatory 24-hr antroduodenal manometry) no organic or functional cause for their

Pt	Excessive Belching	Bloating	Abdominal Distension	Flatulence	Constipation	Abdominal Pain	Epigastric Pain	Reflux symptoms	History of surgery
1		x			**P**	x			
2		x	x		**P**	x			x
3	**P**	x	x				x	x	
4	x	x	x		**P**	x	x		
5	**P**								
6		x	x	x	**P**		x		x
7		x	x	x	**P**	x			x
8		x	x	x	**P**	x			
9		x	x	**P**	x	x			
10	**P**	x							
Total (n)	4	9	7	4	7	6	3	1	3

Table 1 Individual (predominant) symptoms. x: symptom is present; **P**: predominant symptom

symptoms was found. It is likely that the increased amounts of intestinal air and abdominal pain have mistakenly been attributed to an ileus or volvulus, and retrospectively, one could suggest that these patients should not have been operated on. Another possibility is that a large collection of intestinal gas could have contributed to the development of complications. This has been described before, mainly in mentally disabled patients [8-10].

The reason why patients swallow air so frequently remains uncertain. Theoretically, patients may swallow in response to the perception of an unpleasant stimulus, as described in patients with reflux symptoms [15]. Because the first phase of the swallowing process starts voluntarily, one could hypothesize that behavioral therapy or speech therapy can be of benefit in these patients and may even prevent complications.

The main goal of our study was to investigate whether true aerophagia, defined as excessive air swallowing, is present in patients with increased intestinal gas collections and if this can be shown using impedance monitoring. It is important to realize that we investigated only a small group of patients. Further research is necessary to elucidate the role of excessive air swallowing in patients with symptoms such as abdominal distention and bloating and the clinical value of 24-hr esophageal impedance monitoring in this entity.

In conclusion, 24-hr esophageal impedance monitoring enables one to document increased rates of air swallowing in patients in who aerophagia was suspected on the basis of symptoms of excessive amounts of intestinal gas. This is the first study that provides objective evidence for the existence of aerophagia, or excessive air swallowing.

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Speech therapy in patients with excessive supragastric belching - a pilot study

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ABSTRACT

Background & aim: In patients whose main symptom is excessive belching supragastric belching appear to be the predominant mechanism. This belch pattern is characterized by a rapid influx of air into the oesophagus, immediately followed by rapid air expulsion. The rate at which supragastric belching occurs is influenced by attention and distraction, suggesting a behavioural disorder and speech therapy may be of benefit in these patients.

Methods: In 17 consecutive patients with excessive belching, concurrent impedance monitoring and high-resolution manometry was performed to ascertain the mechanism of belching. Patients with supragastric belches were referred to a speech therapist, who was familiar with the concept of supragastric belching. Before and after treatment by the speech therapist, patients filled out a VAS scale regarding the severity of their symptoms.

Results: In all patients supragastric belches were identified with impedance monitoring. Eleven patients were referred to a speech therapist, 6 patients were not able or willing to undergo repetitive treatments. Eleven patients completed treatment by the speech therapist consisting of 10 (8-10) sessions. Overall, the VAS scales showed a significant improvement of the severity of symptoms ($p<0.05$). Six of the 11 patients reported a large decrease (>30%) of their symptoms and 4 patients reported a modest decrease (<30%). In 1 patient the VAS scores indicated an increase in symptoms.

Conclusion: Speech therapy performed by a well-informed speech pathologist leads to a significant symptom reduction in patients with excessive supragastric belching. This is the first study indicating benefit of a treatment for excessive belching.

INTRODUCTION

Belching is a physiological mechanism through which air or gas can be vented from the stomach. This occurs through a relaxation of the lower oesophageal sphincter (LOS), triggered by a vago-vagal reflex induced by distension of the proximal stomach. This relaxation is known as a transient lower oesophageal sphincter relaxation (TLOSR) and acts as a protective mechanism to prevent the stomach from excessive dilatation [1-3].

Patients who present with excessive belching as their predominant symptom are often diagnosed with 'aerophagia' because they are believed to swallow air too frequently or in too large quantities [4]. It has been shown however that these patients do not swallow air too frequently and do not have large amounts of intragastric air, or an increased incidence of TLOSRs [5]. Instead, a typical pattern is observed in the impedance tracings. This pattern is known as supragastric belching and is characterized by a rapid influx of air, followed by a rapid air expulsion, often within one second. Recent research has elucidated two manometrically distinct mechanisms through which the air can flow into the oesophagus. First, air can be pushed into the oesophagus by a pharyngeal contraction, marked by a rise in pharyngeal pressure. The second observed pattern is characterized by a negative intrathoracic pressure ('air sucking').

In addition, it has been shown that the frequency of this supragastric belching can be influenced by attention and distraction, indicating that this type of belching can be considered a behavioural disorder [6]. Because the self-induced nature of supragastric belching, behavioural therapy or speech therapy may be of benefit in patients with excessive supragastric belching.

The aim of our study was to assess the effect of speech therapy in patients with excessive supragastric belching.

METHODS

Patients and study protocol

Included in this study were consecutive patients with excessive belching as dominant symptom presenting between August 2006 and August 2008 at the out-patients clinics of the participating hospitals.

Patients filled in a standard questionnaire regarding gastrointestinal symptoms based on the Rome III criteria for functional dyspepsia and irritable bowel syndrome and on the Montreal classification for gastro-oesophageal reflux disease (GORD) [4, 7, 8].

All patients underwent stationary combined impedance monitoring and high-resolution manometry to assess the number of supragastric belches and the mechanism that resulted in the influx of air.
After an overnight fast, a manometry catheter was introduced transnasally. After correct positioning of the manometry catheter, an impedance catheter was introduced and placed based on the manometrical findings. The stationary measurement consisted of a 10-minute adaptation period, followed by a 30-minute preprandial and a 1-hour postprandial period. A standardized solid meal (505 kCal) was consumed within 30 minutes (McDonald's Quarter Pounder). During the measurement, patients remained in upright position. Pre- and postprandial recordings were performed as patients may experience more symptoms in the postprandial period.
In the patients who presented after August 2007, ambulatory 24-h pH-impedance monitoring was performed immediately after the stationary measurement. Before the study, acid secretion inhibitory drugs were discontinued for 7 days.
When supragastric belches were identified patients were referred to a well-informed speech language pathologist for treatment.

Stationary combined impedance monitoring and high-resolution manometry
A 17-channel water-perfused manometry catheter was used for manometric recording. In addition to sideholes at 5-cm intervals, additional sideholes were located at 1-cm intervals straddling the LOS and upper oesophageal sphincter (figure 1).
All sideholes were perfused at 0.08 ml/min using a pneumohydraulic perfusion system (Dentsleeve Pty ltd, Wayville, South Australia). Pressures were measured with external pressure transducers (Abbott, Sligo, Ireland) and stored in two digital dataloggers (Medical Measurements Systems, Enschede, The Netherlands), using a sample frequency of 20 Hz.
After localization of the LOS, an impedance catheter (Aachen University of Technology, FEMU, Aachen, Germany) with 10 recording segments was introduced with the most distal recording segment located 0-2 cm above the upper border of the LOS. The other recording segments were located as shown in figure 1. Impedance signals were stored in a digital system (Solar, Medical Measurements Systems, Enschede, The Netherlands) recorded with a sample frequency of 50 Hz.

Ambulatory 24h pH-impedance monitoring
After the stationary measurement, ambulatory 24-h pH-impedance monitoring was performed in a subset of patients. For this measurement, a combined pH-impedance catheter (Versaflex, Alpine Biomed, Fountain Valley, California, USA) with 6 impedance recording segments (located at 2-4 cm, 4-6 cm, 6-8 cm, 8-10 cm, 14-16 cm and 16-18 cm above the upper border of the LOS) and an antimony pH-electrode (located at 5cm above the upper border of the

LOS) was used. The impedance and pH signals were stored in a digital datalogger (Ohmega, Medical Measurements Systems, Enschede, The Netherlands) using a sample frequency of 50 and 1 Hz, respectively.

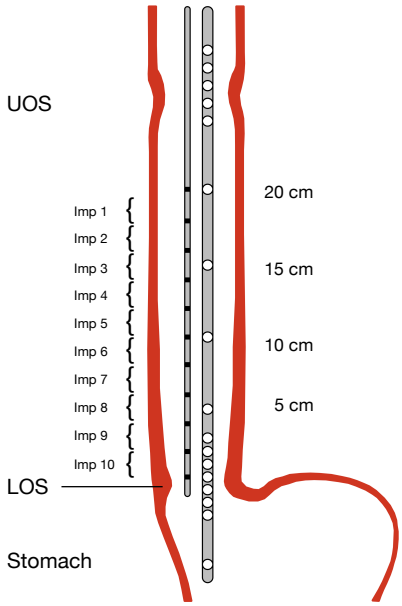

Figure 1 Schematic representation and localization of the impedance (left) and manometry (right) catheter used in the stationary measurement. The impedance (left) catheter was placed with the most distal recording segment (Imp 10) at 0-2 cm above the upper border of the LOS. The manometry catheter has additional sideholes at 1-cm intervals straddling the LOS and UOS. LOS: lower oesophageal sphincter; UOS: upper oesophageal sphincter; Imp: impedance recording segment

Patients were instructed to consume 3 meals and 4 beverages at fixed times, and to note the period spent in recumbent position, as well as reflux symptoms experienced during the measurement.

In order to obtain objective parameters after speech therapy, all patients were asked to undergo a second 24 hour pH-impedance measurement after completion of the speech therapy sessions.

Speech therapy

Patients who exhibited supragastric belches during the stationary measurement were referred to a speech and language pathologist (SLP). The SLP was familiar with the concept of supragastric belching. Before the start of the therapy, the SLP was informed about the manometrical mechanism (pharyngeal contraction and/or air sucking) through which the influx of air during supragastric belches was brought about in each patient.

The therapy focused on explanation and on creating awareness of the belching mechanism. The first step consisted of a description of the behaviour that caused the injection or sucking of air (increased pharyngeal pressure or reduced intrathoracic reduced pressure, respectively). During both periods the patient briefly closed his or her glottis at rest (non speech), accompanied by tight closing of the mouth. As soon as this behaviour was understood, the patient was trained to refrain from these glottal closures and to acquire a normal fluent breathing pattern without these moments of closure. This was practiced by conventional breathing and vocal exercises. As early as possible in the therapy attention on belching was moved to attention on the periods of tight glottal and mouth closure. The cognitive process was regarded as an important aspect in the therapy.
At the beginning and at the end of the speech therapy, patients filled in a 6-item VAS scale regarding the severity of their symptoms (see below). Speech therapy treatment was discontinued after resolution of the patient's symptoms or after 10 one-hour treatment sessions.

Data analysis
In the impedance tracings, reflux episodes were identified and classified according to previously described criteria into liquid, mixed liquid-gas and pure gas reflux episodes [9]. Gastric belches were defined as air-containing reflux episodes (gas and mixed gas-liquid reflux episodes) with the air component reaching the most proximal recording segment [10].
Swallows were defined as a drop in impedance moving in aboral direction. An air swallow was defined as a swallow accompanied by an increase in impedance (≥1000 Ω) in the most distal recording segment [11].
Supragastric belches were defined as a rapid and pronounced rise in impedance (≥1000 Ω) moving in aboral direction, followed by a rapid return to baseline in opposite direction [5].
Concurrent high-resolution manometry was used to assess the mechanism of the influx of air during each supragastric belch. Influx of air into the oesophagus was either induced by creating a negative intrathoracic pressure ('air-sucking') or by an increase of pharyngeal pressure ('air-pushing'), both shortly before the influx of air [5, 6].
High-resolution manometry allowed detection of transient lower oesophageal sphincter relaxations, as defined by previously published criteria [12, 13].
Ambulatory pH-impedance tracings were analyzed manually and reflux episodes were detected as described above. Oesophageal acid exposure was calculated as the percentage of time with a pH below 4 at 5 cm above the upper border of the LOS. Swallows and air swallows were identified using the impedance tracings as described previously [11].
Symptom association analysis was performed using the symptom index (SI) and symptom association probability (SAP) [14, 15]. A positive relationship between reflux episodes and symptoms was defined as SI≥50% and SAP≥95%.

Before and after the speech therapy, patients filled in a short questionnaire (6-item VAS scale) regarding the severity of their belching symptoms. The items focused on severity of the symptoms, the daily inconvenience caused by frequent belching, interference with normal functioning and level of control of belching. The following items were scored: 1. How bothering do you experience your symptom of excessive belching 2. How bothering do you think your environment experiences your excessive belching? 3. Can you suppress belching? 4. Does excessive belching hamper your work/daily activities? 5. Are your social activities hampered by excessive belching? 6. Do you experience any level of control over your excessive belching? All six items (scored in millimetres) were cumulated and compared before and after the treatment sessions.

Major improvement was considered as a >30% decrease of symptom severity. Minor improvement was defined as a 0-30% decrease of symptom severity. The 30% threshold for decrease of symptom severity was considered to be clinically relevant.

Statistical analysis

Throughout the manuscript, data are presented as mean ± SEM (or SD as described otherwise) or median and interquartile range. Reflux episodes and swallowing frequencies during the pre- and postprandial recording period were compared using the paired Student *t*-test. VAS scores (cumulative score of a 6-item questionnaire, in millimetres) at the start and end of the therapy were compared using Wilcoxon signed rank test. Differences were considered statistically significant when $p \leq 0.05$.

RESULTS

Patients

Seventeen consecutive patients (age: 58.1 ± 12.3y (SD); 8 females) with excessive belching as primary and presenting symptom were recruited between August 2006 and August 2008. Patients had symptoms of excessive belching for a mean period of 2.1 ± 1.4 years (SD). None of the included patients had a history of gastrointestinal surgery.

Five out of the 17 patients reported no other symptoms than excessive belching. Besides excessive belching, 9 patients reported symptoms suggestive of GORD, 3 patients reported symptoms suggestive of functional dyspepsia and 6 patients reported symptoms suggestive of irritable bowel syndrome.

Stationary impedance and high-resolution manometry

In all 17 patients supragastric belches were identified as the dominant mechanism of belching. Supragastric belches occurred at a frequency of 32 (8-68)/hr pre- and 41 (28-191)/hr postprandially (p=0.18).

In 14 patients a decrease of intrathoracic pressure was observed prior to the influx of air, indicating that air was sucked into the oesophagus (figure 2A). An increase in pharyngeal pressure prior to the influx of air was observed in 2 patients (air pushing)(figure 2B). In 1 patient both patterns were observed.

Neither the numbers of mixed liquid-gas reflux episodes, gas reflux episodes or gastric belches, nor the number of swallows and air swallows differed between the pre- and postprandial stationary recording period. After the meal, patients had more TLOSRs and more liquid reflux episodes compared to the preprandial recording period (table 1).

Figure 2A

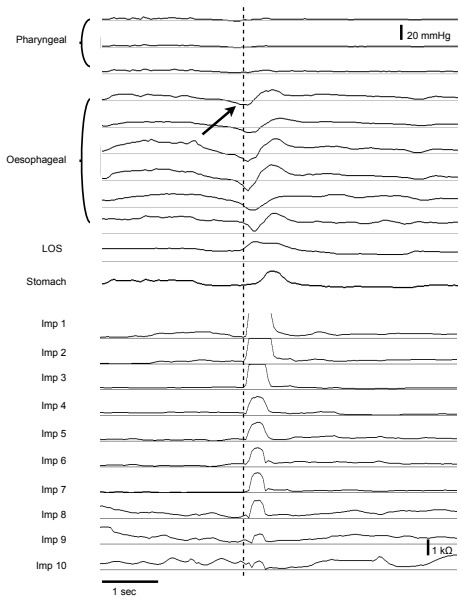

Figure 2B

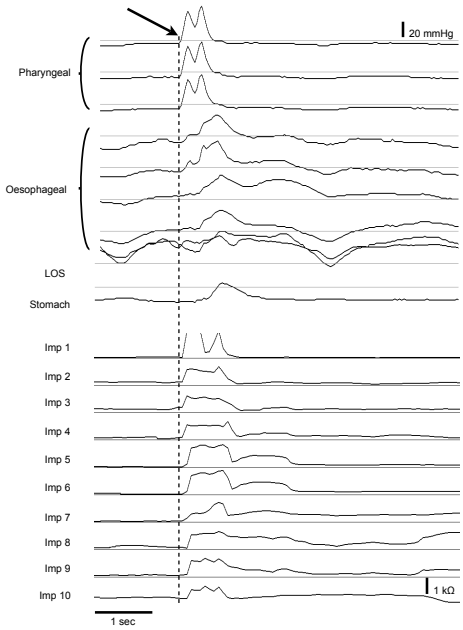

Figure 2 Simultaneous impedance and manometric recordings during a supragastric belch. Two patterns were observed: A) a decrease in intrathoracic pressure (arrow) preceded the influx of air and was followed by an increase of pressure in all channels expelling intra-oesophageal air ('air-sucking') and B) a rise in pharyngeal pressure (arrow), not followed by a peristaltic contraction wave, preceding the influx of air ('air-pushing') into the oesophagus.

	Preprandial (/h)	Postprandial (/h)	p value
Number of supragastric belches	32 (8-68)	41 (28-191)	NS
Number of liquid reflux episodes	4 ± 1	7 ± 2	<0.05
Number of mixed liquid-gas reflux episodes	4 ± 2	3 ± 1	NS
Number of gas reflux episodes	2 ± 1	1 ± 0	NS
Number of gastric belches	5 ± 3	5 ± 1	NS
Number of swallows	91 ± 8	98 ± 16	NS
Number of air swallows	34 ± 10	25 ± 5	NS
Number of TLOSRs	4 ± 0	5 ± 1	<0.05

Table 1 Median (interquartile range) number of supragastric belches, and mean (± SEM) number and type of reflux episodes, gastric belches, swallows, air swallows and TLOSRs per hour during the pre- (30 minutes) and postprandial (1 hour) recording period. TLOSRs: Transient lower oesophageal sphincter relaxations

Ambulatory 24-h pH-impedance monitoring

In 7 consecutive patients an ambulatory 24-h pH-impedance monitoring was performed with a net recording period of 21.9 ± 0.2 hour. During this period a total of 141 (122-1356) supragastric belches were identified with 99 (98-100)% occurring in upright position.

Swallows and air swallows were present at rates of 625 ± 110 /24 hr and 199 ± 55 /24 hr, respectively.

In these patients, 39 ± 5 liquid reflux episodes, 28 ± 5 mixed liquid-gas reflux episodes and 22 ± 8 gas reflux episodes were identified. The number of gastric belches (air-containing reflux episodes with the impedance rise reaching the proximal impedance recording segment) was 50 ± 14 /24 hr. Total oesophageal acid exposure time was 6.0 ± 2.5% (upright: 7.6 ± 2.7%; supine: 2.8 ± 2.6%).

Three out of the 7 patients reported reflux symptoms (epigastric pain and chest pain) during the 24-h recording period. Symptom association analysis resulted in a negative SI (SI<50%) and SAP (<95%) in these patients.

After completion of the speech therapy sessions, only 2 patients agreed to undergo a second 24-h pH-impedance measurement. Both patients had a significant symptom reduction (> 30%) after the speech therapy sessions. In these patients, the number of supragastric belches per 24 hour decreased from 164 and 150 to 6 and 19, respectively.

Speech therapy

Out of the 17 investigated patients, 11 patients were referred to the SLP. The other 6 patients were not able (n=4) or willing (n=2) to comply with repetitive treatment sessions. All 11 patients referred completed treatment in 10 (8-10) therapy sessions.

The VAS scores before and after the treatment sessions are shown in figure 3. Overall, VAS scores decreased significantly (34 (9-73)%) after therapy sessions (before: 420 (214-555) mm vs. after 228 (79-436) mm; $p<0.05$).

In 6 out of the 11 patients speech therapy resulted in major symptom reduction, 4 patients had a modest (0-30%) symptom improvement. One patient reported an increase (50%) in symptoms after the speech therapy sessions.

No differences were found between responders and non-responders with regard to sex and age.

Only 1 out of the 3 patients who pushed air into the oesophagus (pharyngeal contractions) was treated by the SLP. This patient reported a 48% decrease of symptoms after the speech therapy.

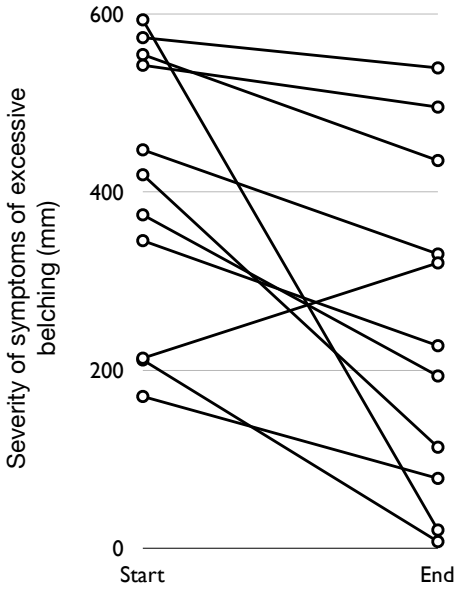

Figure 3 Severity of symptoms of excessive belching (cumulative VAS scores, in mm) of each patient at the start and end of treatment by the speech language pathologist

DISCUSSION

Although every gastroenterologist is confronted occasionally with patients who complain about frequent belching, little research has been done in this field. Even less is known about the treatment strategies for belching disorders, and both patients and physicians are frustrated by this lack of information. Behavioural therapy and speech therapy have been proposed as treatment options in patients with excessive belching. However apart from case reports, little is known about the results of these treatment strategies [16-18].

This is the first study that describes an effective therapy for excessive belching. Although the speech therapy was not equally successful in every patient, a significant number of patients (55%) reported major symptom reduction after the treatment sessions.

All patients who presented with isolated excessive belching exhibited supragastric belches during the stationary measurement. In addition, very large numbers of supragastric belches were observed during the ambulatory measurement. In some patients incidences exceeded 1300 supragastric belches per day, reflecting the severity of the patients' symptoms. In agreement with a previous study, these patients did not exhibit excessive numbers of (air) swallows or TLOSRs, indicating that (air) swallowing is not the cause of their symptoms [5].

Interestingly, patients had increased numbers of liquid-containing reflux episodes. This may be explained by the observation that supragastric belches can elicit reflux episodes [19]. Only 3 patients exhibited (atypical) reflux symptoms during the ambulatory measurement, and none of these patients had a good relationship between reflux symptoms and reflux episodes, underlining that these patients are different patients than reflux patients.

In our study patients were treated by a speech and language pathologist who was familiar with the supragastric belching disorder. Therapy was focused on awareness and regaining control of supragastric belching and normalizing breathing patterns. As the therapy concerns mostly a behavioural change, it may be argued that therapy by a behavioural therapist may be of benefit as well. Because speech therapists have much knowledge in pharyngeal and laryngeal anatomy and function, we preferred treatment by an expert in this field.

The question remains why patients exhibit these supragastric belches and in such high frequencies. We hypothesize that these patients start with inducing supragastric belches consciously, probably in response to an unpleasant sensation, and lose control afterwards. Theoretically, this unpleasant sensation may be some kind of discomfort associated with functional oesophageal disorders, dyspepsia or irritable bowel syndrome. This is supported by the significant number of our patients reporting other gastrointestinal symptoms.

This study has some limitations. First, the study is hampered by a relatively low number of patients with isolated excessive belching, and it does not include a control group. However, it is not likely that the belching symptoms would resolve spontaneously as most patients had symptoms for several years. Second, a relatively large group of patients dropped out of the

study because they were not able or willing to comply with repetitive visits to the speech therapist. In most cases this was due to long travel distances that are inherent to tertiary referrals. Finally, the effect of the speech therapy was assessed using VAS scales regarding the patients' symptoms only. No objective parameters were obtained. However, this is the first study that investigated the effect of speech therapy systematically in a group of patients. Symptom reduction by speech therapy is feasible in the majority patients with excessive supragastric belching.

In conclusion, this is the first study that describes speech therapy as treatment for supragastric belching. In well-motivated patients this approach can lead to a significant symptom reduction. Further research on the effect of speech therapy in patients with excessive supragastric belching is warranted.

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Summary

11

In the general introduction (**chapter 1**) we provide an overview of the definition of gastro-oesophageal reflux disease. The current knowledge about the pathophysiology of gastro-oesophageal reflux disease is reviewed. The emphasis lies on the identified and proposed mechanisms that lead to the development of symptoms or complications in patients with GORD. Furthermore, the current diagnostic and therapeutic strategies are discussed. Finally, the physiology of belching is explained and the current knowledge about different belch patterns and belching disorders is reviewed.

In **chapter 2**, the characteristics of antimony, ISFET and glass pH electrodes are investigated both under in vitro and in vivo conditions. In an in vitro model the response time, sensitivity and drift of an antimony, ISFET and glass pH electrode were assessed. Fifteen patients with reflux symptoms underwent 24-h pH monitoring off PPI therapy using antimony, ISFET and glass pH electrodes simultaneously. In contrast with antimony and glass electrodes, calibration temperature did not affect the accuracy of ISFET electrodes. During in vivo experiments, antimony and ISFET electrodes resulted in significantly lower acid exposure times than glass pH electrodes. Significant differences between acid exposure times are obtained with antimony, ISFET and glass pH electrodes. From the results of the vitro measurements, we conclude that ISFET electrodes result in the most accurate in vivo measurements of acid exposure time.

The manual analysis of ph-impedance recordings for detection of gastro-oesophageal reflux episodes requires expertise and is time-consuming. In **chapter 3** we assess the accuracy of newly developed software for detection of reflux episodes. Results of computer analysis of 24-h oesophageal impedance recordings from 10 patients were compared with the results of manual analysis performed by 3 independent investigators. In addition, 24-h impedance tracings of 60 consecutive patients with reflux symptoms were analyzed manually by one investigator and were also analyzed using computer software. Computer analysis resulted in both lower sensitivity and specificity compared to the manual analysis of the investigators. Despite the lower sensitivity and specificity, symptom association analysis performed by the computer and a human observer showed concordant results in 83% of the patients. Although not as good as manual analysis by experts, computer analysis can be a helpful tool to identify reflux episodes and to assess the relationship between reflux episodes and symptoms.

In **chapter 4** we describe a study in which the yield of combined pH-impedance monitoring performed both ‘on’ and ‘off’ proton pump inhibitor (PPI) therapy is compared in patients with PPI-resistant symptoms. For this study, 30 patients with typical reflux symptoms despite PPI therapy underwent ambulatory 24-h pH-impedance monitoring twice, once on PPI and once after cessation of PPI therapy. The total number of reflux episodes and proximal extent were

not affected by PPI therapy. On PPI, there were fewer acid reflux episodes while more weakly acidic reflux episodes were identified. In addition, more patients were asymptomatic during the measurement on PPI therapy. Although not statistically significant, symptom association analysis in the measurement off PPI therapy showed a higher yield compared to the measurement on PPI. We conclude that in order to demonstrate or exclude GORD in patients with PPI-resistant symptoms, ambulatory 24-h pH-impedance monitoring should preferably be performed after cessation of PPI therapy. This approach offers the best chance to assess a relationship between symptoms and reflux episodes.

GORD patients often report an increase of their reflux symptoms during stressful situations. In **Chapter 5** we assessed the influence of acute psychological stress on oesophageal acid perception. In 15 healthy volunteers and 10 GORD patients with a positive symptom-reflux association an oesophageal acid perfusion test was performed, once with and once without the presence of an acute psychological stressor (a modified IQ test). The time from onset of the acid infusion to first acid perception, discomfort and pain was noted. In both healthy volunteers and GORD patients, the time to first perception, discomfort, or pain did not differ significantly between both measurements. Systolic blood pressure rose significantly during the stress task in both groups. We conclude that neither in healthy volunteers nor in GORD patients, acute psychological stress induced by an IQ test increased oesophageal acid perception.

Although patients with mucosal lesions have a higher oesophageal acid exposure, it is unclear whether patients with various degrees of oesophageal damage have different reflux patterns. In **chapter 6** we compare the characteristics of reflux episodes in controls and in patients with various degrees of oesophagitis and Barrett's oesophagus. Ambulatory 24-hour impedance-pH tracings were analyzed from healthy volunteers, patients with non-erosive reflux disease (NERD), patients with grade A oesophagitis, grade B oesophagitis, grade C or D oesophagitis and patients with a short segment of Barrett's metaplasia. A higher number of acid and weakly acidic reflux episodes was observed in the patients with increasing degrees of oesophagitis and Barrett's oesophagus. The proportion of acidic or alkaline reflux episodes was similar all groups. Compared with normal values, only 40% of the patients with NERD had an abnormally high total number of reflux episodes. In the patients with severe oesophagitis a significantly higher percentage of reflux episodes reached the proximal oesophagus compared to the patients with Barrett's oesophagus. These data show that with increasing degrees of oesophagitis, patients have more reflux episodes. A large overlap between the groups exists making comparison to normal values of limited relevance. In patients with short segment Barrett's oesophagus fewer reflux episodes reach the proximal oesophagus which might explain their low sensitivity to reflux.

In **chapter 7** we investigated the mechanism that leads to an increased swallowing frequency in GORD patients. In 34 patients with typical reflux symptoms oesophageal 24-h pH-impedance monitoring was performed twice, once off and once on PPI therapy. The number of reflux episodes and number of swallows and air swallows was evaluated. The symptom association probability (SAP) was used to distinguish patients with a good relationship between symptoms and reflux episodes (SAP+) from those who had not (SAP-). We showed that the swallowing frequency decreased in the SAP+ patients during PPI therapy. In contrast, in the SAP- patients the incidence of swallows was not influenced by the PPI therapy. These data support the hypothesis that the increased incidence of swallows in GORD is brought about by responses to perceived reflux events.

Supragastric belching is a distinct belch pattern found in patients with excessive belching. It is unclear whether supragastric belches occur in patients with reflux symptoms. In **chapter 8** we describe a study in which we investigate the belching patterns in patients with reflux symptoms and healthy volunteers. Fifty consecutive patients with typical reflux symptoms and 10 healthy volunteers underwent ambulatory 24-h pH-impedance monitoring. Supragastric belches were identified in approximately 50% of both patients with reflux symptoms and healthy volunteers. Patients with reflux symptoms had higher incidences of supragastric belches than healthy controls. Moreover, a temporal association between supragastric belches and reflux episodes was identified in 48% of the supragastric belches in patients with reflux symptoms. Two different association patterns were observed: 30% of the supragastric belches occurred immediately prior (<1 second) to the onset of the reflux episode. In 18% the supragastric belch occurred during the reflux episode, with the onset 4-10 seconds after the start of the reflux episode. We conclude that supragastric belches occur more frequently in patients with typical reflux symptoms than in healthy subjects. These belches often occur in close association with reflux episodes. Our findings suggest that supragastric belching elicits reflux in some cases and is the patient's response to an unpleasant esophageal sensation in others.

In **chapter 9**, swallowing and air swallowing frequencies were investigated in patients with an excessive amount of intestinal gas, which is thought to result from excessive air ingestion, or aerophagia. Ambulatory 24-hr pH-impedance monitoring was performed in 10 patients in whom excessive amounts of intestinal gas were visualized on plain abdominal radiograms. The most common symptoms among these patients were bloating, abdominal distention and constipation. Only three patients reported excessive belching and one patient reported flatulence as their predominant symptom. During the 24-h measurement, all patients showed high incidences of air swallows and gastric belches while normal swallowing frequencies were

found. This study presents objective parameters confirming the existence of a phenomenon of excessive air swallowing or aerophagia using esophageal impedance monitoring.

In **chapter 10** we investigated the effect of speech therapy in patients with excessive supragastric belching. Concurrent impedance monitoring and high-resolution manometry was performed in patients with excessive belching. Patients with supragastric belches were referred to a speech therapist, who was familiar with the concept of supragastric belching. In more than 50% of the patients, speech therapy resulted in significant improvement of the severity of symptoms. Therefore, we conclude that speech therapy performed by a well-informed speech therapist leads to a significant symptom reduction in patients with excessive supragastric belching.

Nederlandse samenvatting

In **hoofdstuk 1**, de algemene inleiding, wordt de definitie en de huidige kennis van de pathofysiologie van gastro-oesofageale refluxziekte beschreven. De nadruk ligt hierbij op de complexe relatie tussen verschillende mechanismen die leiden tot het ontstaan van klachten en complicaties van gastro-oesofageale refluxziekte. Ook de diagnostiek en (nieuwe) therapeutische inzichten worden besproken. Daarnaast gaan we in op de verschillende vormen van eructatie oftewel boeren. Zowel de mechanismen die leiden tot het laten van normale maagboeren als de mechanismen betrokken bij het totstandkomen van (overmatig) supragastrisch boeren worden behandeld.

Hoofdstuk 2 behandelt de eigenschappen van verschillende pH-elektroden die gebruikt worden voor 24-uurs pH-metingen. In een in vitro model worden de responstijd, de sensitiviteit en drift van zowel antimoon-, ISFET- als glas-elektroden onderzocht. Vervolgens werd bij een 15-tal patiënten een gecombineerde meting met de 3 verschillende pH-elektroden verricht. In tegenstelling tot bij de antimoon- en glas-elektrode, beïnvloedde de calibratietemperatuur de nauwkeurigheid van de ISFET-elektrode niet. Tijdens de in vivo metingen resulteerden de antimoon- en ISFET-elektroden in lagere 'acid exposure times' in de slokdarm dan glas-elektroden. Grote verschillen in acid exposure times werden verkregen met de 3 geteste pH-elektroden. Uitgaande van de in vitro metingen concluderen we dat de ISFET-elektrode resulteert in de meest betrouwbare meting.

De handmatige analyse van pH-impedantie-signalen voor het detecteren van gastro-oesofageale reflux vergt ervaring en is tijdrovend. In **hoofdstuk 3** wordt de nauwkeurigheid onderzocht van nieuw ontwikkelde software die automatisch refluxepisodes detecteert. De metingen van 10 patiënten die een 24-uurs impedantiemeting van de slokdarm hadden ondergaan, werden zowel automatisch geanalyseerd door de nieuwe software als handmatig door 3 onafhankelijke experts. Aanvullend werden de metingen van 60 patiënten met refluxklachten geanalyseerd door de computersoftware en door een van de experts. Analyse door de computersoftware had een lagere sensitiviteit voor de detectie van refluxepisodes en resulteerde in meer vals-positieven dan de handmatige analyse. Ondanks deze lagere sensitiviteit en specificiteit resulteerde de analyse van de associatie tussen symptomen en refluxepisoden bij 83% van de patiënten in gelijkaardige uitkomsten. Deze bevindingen laten zien dat computersoftware, hoewel minder betrouwbaar dan handmatige analyse door experts, een handig hulpmiddel kan zijn voor de detectie van refluxepisodes en voor de identificatie van een relatie tussen klachten en refluxepisodes.

De meest voorkomende reden voor het falen van therapie van refluxziekte met een protonpompremmer (proton pump inhibitor, PPI) is een onterechte diagnose van refluxziekte. In **hoofdstuk 4** vergelijken we in patiënten met therapieresistente refluxklachten de opbrengst

van een pH-impedantiemeting uitgevoerd zowel tijdens het gebruik als na het staken van PPI. Voor deze studie ondergingen 30 patiënten twee keer een 24-uurs pH-impedantiemeting, één met en één zonder het gebruik van PPI. De volgorde van de metingen werd gerandomiseerd. De resultaten tonen dat het totaal aantal refluxepisodes en de uitbreiding van het refluxaat naar proximaal niet beïnvloed wordt door PPI-therapie. Alleen de zuurtegraad van de refluxepisodes verandert tijdens het gebruik van PPI: tijdens PPI-gebruik worden meer zwak-zure en minder zure refluxepisodes gevonden dan zonder het gebruik van een PPI. Tijdens de meting met PPI waren meer patiënten asymptomatisch, waardoor er geen relatie met de klachten onderzocht kon worden. Hieruit concluderen wij dat een 24-uurs pH-impedantiemeting bij patiënten met therapieresistente refluxklachten de grootste diagnostische opbrengst heeft wanneer deze uitgevoerd wordt na het staken van PPI-behandeling. Een meting zonder PPI geeft de grootste kans dat een verband tussen klachten en refluxepisodes aangetoond of uitgesloten kan worden.

Hoofdstuk 5 handelt over de relatie tussen refluxklachten en stress. In dit hoofdstuk beschrijven we een studie naar de invloed van een acute psychologische stressor op de gevoeligheid van de distale slokdarm voor zuur. Bij 15 gezonde vrijwilligers en 10 patiënten met refluxziekte werd twee keer zuur in het onderste deel van de slokdarm gebracht, eenmaal met en eenmaal zonder de aanwezigheid van een acute psychologische stressor (een aangepaste IQ test). De volgorde van deze metingen werd gerandomiseerd. De primaire uitkomstmaat was de tijd van start van zuurinfusie tot de eerste gewaarwording, onaangenaam gevoel en pijn. Bij zowel de gezonde vrijwilligers als de patiënten met refluxziekte werd de tijd tot eerste gewaarwording, onaangenaam gevoel of pijn niet beïnvloed door een acute psychologische stressor. In beide groepen nam de systolische bloeddruk toe tijdens de IQ test, suggererend dat de aangeboden stressor effectief was. We concluderen dat acute psychologische stress uitgelokt door een aangepaste IQ test de gevoeligheid van de slokdarm niet beïnvloedt, noch bij gezonde vrijwilligers noch bij patiënten met refluxziekte.

Hoewel bij patiënten met schade aan de slokdarm over het algemeen een grotere blootstelling van de slokdarm aan zuur wordt aangetroffen, is het niet bekend of patiënten met verschillende graden van slokdarmschade ook verschillende refluxkarakteristieken hebben. In **hoofdstuk 6** vergelijken we de refluxkarakteristieken van gezonde vrijwilligers met patiënten met verschillende graden van slokdarmontsteking (oesofagitis graad A-D) en kort-segment Barrett-slokdarm, dit door middel van ambulante 24-uurs pH-impedantiemetingen. Grotere aantallen van zure en zwak-zure refluxepisodes werden aangetoond bij patiënten met toenemende graden van oesofagitis en Barrett-slokdarm. Het percentage zure en zwak-zure reflux episodes was gelijk in alle groepen. Vergeleken met controles heeft slechts 40% van de patiënten met refluxziekte zonder zichtbare slokdarmafwijkingen een verhoogd aantal

refluxepisodes. Bij patiënten met ernstige graden van refluxoesofagitis reiken refluxepisodes vaker tot het bovenste deel van de slokdarm dan bij patiënten met een Barrett-slokdarm. We concluderen dat patiënten met ernstigere graden van oesofagitis meer refluxepisodes hebben. Er bestaat echter een grote overlap tussen de verschillende groepen, wat maakt dat vergelijking met normaalwaardes van beperkt belang is. Bij patiënten met Barrett-slokdarm reiken refluxepisodes minder vaak tot in het proximale deel van de slokdarm. Deze bevinding verklaart mogelijk dat patiënten met Barrett-slokdarm minder gevoelig zijn voor reflux.

In **hoofdstuk 7** onderzochten we het mechanisme dat leidt tot een toename van de slikfrequentie bij patiënten met refluxziekte. Bij 34 patiënten met refluxklachten werd twee keer een 24-uurs pH-impedantiemeting uitgevoerd, eenmalig met en eenmalig zonder het gebruik van een PPI. Het aantal slikken en het aantal luchtslikken werd geëvalueerd. Door middel van de symptom association probability (SAP) werden patiënten met een duidelijke relatie tussen refluxklachten en refluxepisodes (SAP- positief) onderscheiden van patiënten die deze relatie niet hadden (SAP-negatief). Bij de SAP-positieve patiënten daalde de slikfrequentie tijdens het gebruik van een PPI. Dit in tegenstelling tot de patiënten met een negatieve SAP, waarbij het aantal slikken niet beïnvloed werd door PPI. Deze bevindingen ondersteunen de hypothese dat de verhoogde slikfrequentie bij patiënten met refluxziekte veroorzaakt wordt als reactie op de waargenomen refluxepisodes.

Hoofdstuk 8 handelt over het voorkomen van supragastrisch boeren bij patiënten met refluxklachten. Supragastrisch boeren is een typisch boerpatroon dat voor het eerst werd aangetoond bij patiënten met overmatig boeren. In dit hoofdstuk onderzoeken we de verschillende boerpatronen bij gezonde vrijwilligers en patiënten met refluxklachten. Vijftig patiënten met refluxklachten en 10 gezonde vrijwilligers ondergingen een ambulante 24-uurs ph-impedantiemeting. Bij ongeveer 50% van de patiënten en gezonde vrijwilligers werden supragastrische boeren aangetoond. Bij patiënten met refluxklachten werden hogere aantallen supragastrische boeren gevonden dan bij gezonde vrijwilligers. Daarnaast werd in 48% van de gevonden supragastrische boeren een relatie met een refluxepisode aangetoond. Twee verschillende patronen werden geobserveerd: 30% van deze supragastrische boeren begon <1 seconde voor de start van de refluxepisode. De overige 18% van de supragastrische boeren vonden plaats tijdens de refluxepisode, 4-10 seconden na de start van de refluxepisode. De conclusie van dit onderzoek is dat supragastrische boeren vaker voorkomen bij patiënten met refluxklachten dan bij gezonde vrijwilligers, en dat deze vaak een temporele relatie vertonen met refluxepisodes. Deze bevindingen suggereren dat supragastrische boeren refluxepisodes kunnen induceren, en dat ze soms een reactie zijn op een onaangenaam gevoel in de slokdarm.

In **hoofdstuk 9** worden de aantallen slikken en luchtslikken beschreven bij patiënten met overmatige hoeveelheden intestinaal gas, dat mogelijk veroorzaakt werd door overmatig 'lucht eten' of aerofagie. Bij 10 patiënten met overmatige hoeveelheden intestinaal gas, aangetoond op buik-overzicht röntgenfoto's, werd een ambulante 24-uurs pH-impedantiemeting uitgevoerd. De meest voorkomende symptomen van deze patiënten waren een opgeblazen gevoel, een uitgezette, gespannen buik en obstipatie. Slechts drie patiënten presenteerden zich met overmatig boeren en één patiënt met toegenomen flatulentie. Tijdens de 24-uurs pH-impedantiemeting werd bij alle patiënten een verhoogd aantal luchtslikken en gas-vormige reflux (maagboeren) aangetoond, terwijl het aantal normale slikken (zonder lucht) niet was toegenomen. Deze studie toont aan dat met behulp van slokdarm-impedantiemetrie objectieve gegevens kunnen worden verkregen, die het bestaan van het fenomeen overmatig luchtslikken of aerofagie bevestigen.

In **hoofdstuk 10** onderzochten we het effect van logopedie bij patiënten met overmatig supragastrisch boeren. Patiënten bij wie door middel van gelijktijdig uitgevoerde impedantiemeting en hoge-resolutie-manometrie van de slokdarm was aangetoond dat ze leden aan supragastrisch boeren werden verwezen naar een logopediste met speciale kennis van dit fenomeen. Bij meer dan 50% van de patiënten resulteerde logopedische behandeling in een significante verbetering van de symptomen. We concluderen dan ook dat logopedische behandeling door een goed-geïnformeerde logopedist tot een aanzienlijke symptoomvermindering kan leiden bij patiënten met overmatig supragastrisch boeren.

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Chapter 11

Curriculum vitae

Maarten Hemmink werd geboren op 9 juni 1981 in Almelo. In de zomer van 1999 behaalde hij het middelbare schooldiploma op het Noordik te Almelo en begon hierna met de studie Geneeskunde aan de Universiteit Antwerpen. Tijdens de laatste studiejaren deed hij onderzoek op de afdeling Cardiologie en later op de afdeling Gastroenterologie (Prof. M. Michielsen) in het Universitair Ziekenhuis Antwerpen. Op 16 juni 2006 studeerde hij af-met de vermelding grote onderscheiding. Hierna werkte hij als arts-onderzoeker op de Maag-, Darm- en Leverziekten in het Sint Antonius Ziekenhuis te Nieuwegein onder begeleiding van Dr. A.J. Bredenoord, Dr. B.L.A.M. Weusten, Dr. R. Timmer en Prof. A.J.P.M. Smout. In april 2009 begon hij met de vooropleiding interne geneeskunde in het St. Antonius Ziekenhuis (opleider: Dr. A.B. Geers). Op 4 juli 2009 is hij getrouwd met Femke Witjes.