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Reflux Mechanisms in Gerd : Analysis of the role of transient lower esophageal sphincter relaxations

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Citation

Straathof, J. W. A. (2005, October 31). *Reflux Mechanisms in Gerd : Analysis of the role of transient lower esophageal sphincter relaxations*. Retrieved from https://hdl.handle.net/1887/11001

Version:	Corrected Publisher's Version
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Note: To cite this publication please use the final published version (if applicable).

Stellingen

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REFLUX MECHANISMS in GERD:
Analysis of the role of
Transient Lower Esophageal Sphincter Relaxations

1. Er bestaan verschillende typen refluxers: aan de ene kant patiënten met reflux tijdens TLESR en aan de andere kant patiënten met een incompetente gastro-oesofageale overgang. *(dit proefschrift)*

2. De frequentie van TLESR is afhankelijk van de rustdruk van de onderste slokdarm sfincter. *(dit proefschrift)*

3. Distensie van de maag door insufflatie van lucht bij patiënten na complete fundoplicatie kan een beperking van opboeren van lucht aantonen. *(dit proefschrift)*

4. Gastrine verlaagt de onderste slokdarm sfincter rustdruk. *(dit proefschrift)*

5. De toename van onderste slokdarm sfincter druk door bombesine heeft geen invloed op het optreden van TLESR. *(dit proefschrift)*

6. De Sphinctometer registreert TLESR met vergelijkbare resultaten als de Dentsleeve. *(dit proefschrift)*

7. Het optreden van TLESR na laparoscopisch Nissen fundoplicatie is relevant om op een fysiologische manier lucht te ventileren uit de maag. *(dit proefschrift)*

8. De duur van de relaxatie is de meest karakteristieke eigenschap van een TLESR.

9. Ondanks het klinische, sociale en economische belang wordt het opgeblazen gevoel (bloating) substantieel genegeerd, zonder voldoende klinische kwalificatie, bewezen pathofysiologie of effectieve behandeling. *(Gastroenterology 2005; 129:1060-1078)*

10. Patiënten dienen te worden geïnformeerd over het risico van dysphagie na laparoscopische anti-reflux chirurgie. *(Lancet 2000; 355:170-174)*

11. Endoscopische anti-reflux procedures kunnen nog niet alle beloftes waarmaken.

12. Met één zin de hele situatie beschrijven, dat is de kracht van schrijven. *(Godfried Bomans)*

13. Omdat hondenbezitters tijdens het uitlaten aan buurtpreventie doen, zouden zij moeten worden vrijgesteld van gemeentebelasting.

14. Sport is uitsluitend die spelvorm waarbij mensen met elkaar in competitie treden, hetzij als enkeling, hetzij als team, met in achtneming van bepaalde spelregels. *(E.L. Noach)*

J.W.A. Straathof, oktober 2005

REFLUX MECHANISMS IN GERD:

Analysis of the role of

transient lower esophageal sphincter relaxations

Jan Willem Straathof

Reflux mechanisms in GERD:
Analysis of the role of Transient Lower Esophageal Sphincter Relaxations.

Straathof, J.W.A.

Thesis, Leiden University.

ISBN 90-9020065-7

Cover: Esophago-gastric junction.

Printing of this thesis was financially supported by:
Janssen-Cilag B.V., Roche Nederland B.V., Olympus Nederland B.V..

AstraZeneca B.V.; Altana Pharma B.V. .
Part of the studies presented in this thesis was financially supported
by a grant from Maag Lever Darm stichting, the Netherlands.

Printing: PrintPartners Ipskamp.

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REFLUX MECHANISMS IN GERD:

Analysis of the role of transient lower esophageal sphincter relaxations

Proefschrift

ter verkrijging van

de graad van Doctor aan de Universiteit Leiden,

op gezag van de Rector Magnificus Dr. D.D. Breimer,

hoogleraar in de faculteit der Wiskunde en

Natuurwetenschappen en die der Geneeskunde,

volgens besluit van het College voor Promoties

te verdedigen op 31 oktober 2005

klokke 16.15 uur

door

Johannes Wilhelmus Adrianus Straathof

geboren te Eindhoven in 1965

Promotiecommissie

Promotor:	Prof. dr. C.B.H.W. Lamers
Co-promotor:	Dr. A.A.M. Masclee
Referent:	Prof. dr. A.J.P.M. Smout (Universiteit Utrecht)
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Odyssee (Homerus, ~800 v. Chr)

Vertel mij over de man, Muze, de listige, die zeer veel gezworven heeft,
nadat hij de heilige burcht van Troje verwoest had.
Van veel mensen zag hij de steden en leerde hun denkwijze kennen,
veel beproevingen doorstond hij op zee, in zijn hart vechtend
voor zijn leven en de terugkeer van zijn makkers.

Voor Sabine, Dirk Jan, Elianne en Arnout.

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1

INTRODUCTION:

TRANSIENT LOWER ESOPHAGEAL SPHINCTER RELAXATIONS

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Gastro-esophageal reflux

Heartburn, resulting from gastro-esophageal acid reflux, is one of the most common gastro-intestinal symptoms in the general population (1). In the past decades we have gained a better insight into the mechanisms that cause gastro-esophageal reflux (Table 1). Nowadays gastro-esophageal reflux is considered a motility disorder. The main barrier for gastro-esophageal reflux is the esophago-gastric junction. A constant elevated pressure barrier at the esophagogastric junction is maintained by the lower esophageal sphincter (LES) and the crural diaphragm. A hiatal hernia separates the two pressures of lower esophageal sphincter and crural diaphragm and thereby diminishes the synergistic pressure effect reading to a more weak anti reflux barrier (2). The lower esophageal sphincter is able to relax to a pressure equal to intragastric pressure. Short-lasting, swallow-induced relaxations occur during ingestion of liquid or solid food. Prolonged relaxation of LES also occurs. These phenomena are not related to swallowing and have been described as transient lower esophageal sphincter relaxations (TLESRs). There are also other gastrointestinal factors that influence gastroesophageal reflux. The content of the refluxate is of importance for damaging esophageal mucosa. Not only acidic content of the stomach has an erosive effect on the esophagus. Bile mixes with acid after duodenal-gastric reflux. Bile can be measured using fotospectrometry (Bilitec 2000). Patients with Barrett esophagus have excess of biliary duodeno-gastro-esophageal reflux.

Table 1. *Factors involved in the pathogenesis gastro-esophageal reflux*

- Low LES pressure
- Transient LES relaxation
- Hiatal Hernia
- Ineffective esophageal peristalsis
- Delayed gastric emptying
- Content of refluxate
- Saliva
- Esophageal mucosal resistance

Delayed gastric emptying increases gastroesophageal reflux. Not only by increasing the time of intragastric content but also by prolonged gastric distension. High caloric or fat containing meals delay gastric emptying. In the esophagus several mechanisms are involved in clearance of gastroesophageal reflux. Salivary flow can neutralize acidic refluxate. Esophageal motility performs mechanical clearance through primary and/or secondary peristalsis. The last defense mechanism is the esophageal mucosa.

Gastroesophageal reflux may lead to specific symptoms like heartburn, retrosternal pain, regurgitation, belching and dysphagia. Long-lasting gastroesophageal reflux of acidic gastric content may lead to erosive esophagitis, esophageal ulceration, esophageal intestinal metaplasia (Barrett) and even strictures of the esophagus. With endoscopy one is able to objectify the severity of esophagitis. Long-term ambulatory pH-metry is of clinical value in the detection of pathologic reflux in patients with GERD (3; 4). The grade of esophagitis is

correlated to the amount of reflux measured by 24-hour pH metry. Percentage of time with esophageal pH below 4 and number of reflux episodes lasting longer than 5 minutes are the best parameters to indicate the severity of gastroesophageal reflux (5). Combining esophageal pH- and manometry may give information on mechanisms of gastroesophageal reflux.

Transient lower esophageal sphincter relaxations

Transient lower esophageal sphincter relaxations are abrupt decreases of lower esophageal sphincter pressure to intragastric pressure. These relaxations are not related to swallowing. TLESR probably are a mechanism to evacuate excess of intragastric air. Therefore the TLESR may function as a safety valve of the esophagogastric junction. (6; 7). TLESR are identified by esophageal manometry. Water perfused manometry with catheter with small side-hole openings is not adequate, since the position of the catheter orifice at the esophagogastric junction changes during respiration. The standard method for continuous LES registration is the water perfused sleeve device. The sleeve has been first described by Dent in 1978 (8). The water perfused sleeve has been used extensively both in animal as in human studies and is considered as the golden standard technique for lower esophageal sphincter manometry. Miniature devices were able to detect TLESR at very early age (9; 10).

Prolonged recording of LES pressure was possible using a portable manometric system (11). A solid-state catheter has been developed for sphincter manometry. This sphinctometer is not dependent on water perfusion, but it consists of an oil filled cylinder incorporating a solid state transducer. The sphinctometer records LES pressure and identifies TLESR. However due to the technique LES pressures are expressed lower and the LES pressure is dependent on the length of the sleeve exposed to the LES. Because of low LES pressure output, it may prove more difficult to identify TLESR with sphinctometer than with standard sleeve manometry(12; 13).

Holloway described objective criteria for TLESR. Based on analysis in healthy subjects and patients with GERD, TLESR were defined by 1) absence of swallowing for 4 s before to 2 s after the onset of LES relaxation, 2) relaxation rate of $\geq$1 mmHg/s 3) time from onset to complete relaxation of $\leq$10 s and 4) nadir pressure of $\leq$2 mmHg. Prolonged relaxations by multiple swallows are excluded. LES pressure falls that fulfill the last three criteria but have a duration of >10 s can also be classified as TLESR irrespective of timing of LES relaxation to swallowing (14)

LES tracing are analyzed visually in order to detect TLESRs. Although there are precise criteria for TLESR interobserver variation may lead to differences in detection of TLESR. Computer analysis of LES tracings is feasible however computer assisted detection of TLESR until now, has not been satisfactorily accomplished (15; 16).

The frequency of TLESR has been investigated extensively. First, they have been identified in animals, healthy human subject and patient with GERD. Secondly mechanisms that increase TLESR frequency were studied, and third factors that inhibited the frequency of TLESR were studied. Main factors that influence TLESR a summarized in Table 2.

Even a catheter in the pharynx affects TLESR. Subjects with catheters placed via the pharynx have been compared with manometric catheters placed via a gastrostomy tube. The frequency of TLESR was significantly higher with a catheter positioned in the pharynx and therefore it was concluded that the pharynx may mediate the induction of TLESR (17). Stimulation of laryngopharyngeal mechanoreceptors induces LES relaxation but not crural

diaphragm relaxation. (7; 18; 19). Gastro-esophageal reflux was found only during simultaneous LES and crural diaphragm relaxations.

Body posture affects the frequency of TLESR. The frequency of TLESR was higher in the vertical position than in the horizontal position (20-22). Sleep inhibits the frequency of TLESR (23).

The frequency of TLESR varies during the day. Under fasting conditions the frequency of TLESR ranges from 0-3 TLESR per hour. Ingestion of a meal results in an increase in the frequency of TLESR. The frequency of TLESR after meal ingestion is dependent on both the volume and content of the meal. After meal ingestion the number of TLESR may increases to about 3-6 TLESR per hour (24; 25). The frequency of TLESR after a fat containing meal persists for several hours, while after ingestion of a carbohydrate, low fat containing meal the frequency of TLESR more rapidly returns to basal level (26). Another study on composition of the meal reported no significant differences between a balanced meal and a high fat meal, neither in patients with GERD nor in healthy subjects (27)

Gastric distension is a potent trigger of TLESR. Distention of the proximal stomach with intragastric air or an intragastric balloon increases the frequency of TLESR (28). It has been described in dogs that the subcardiac region of the stomach in dogs is primarily responsible for triggering TLESR (29). Intragastric distension with air provoked TLESR with shorter duration than those seen after distension with liquid. At high intragastric pH fewer TLESR occurred compared to low pH. Triggering of TLESR is therefore dependent both on physical and chemical nature of the stimulus (30).

Table 2 *Factors that influence TLESR*

Increase in frequency of TLESR
• Meal composition
• Gastric distension
• Cholecystokinin
• Cholestyramine
Decrease in frequency of TLESR
• Muscarine receptor antagonist (atropine)
• Opiod alkaloid (morphine)
• Nitric-oxide synthase inhibitor (L-NAME; L-NMMA)
• CCK-receptor antagonist (loxiglumide)
• 5HT3 receptor antagonist (ondansetron, granisetron)
• 5HT4 receptor partial agonist (tegaserod)
• GABA-B receptor antagonist (baclofen)
• Body posture
• Sleep
• Fundoplication
• Radiofrequency energy at esophagogastric junction

In asymptomatic recumbent subjects gastroesophageal reflux is related to TLESR rather than to low basal LES pressure (31). The mechanisms of gastroesophageal reflux have repeatedly been evaluated in patients with reflux esophagitis. The predominant reflux mechanism varied in individual patients: some had normal resting LES pressure and reflux that occurred primarily during TLESR, whereas others with low resting sphincter pressure had spontaneous free reflux over a defective or insufficient LES or reflux that occurred during an increase in abdominal pressure (32). It is not clear whether patients with GERD differ from controls with respect to the frequency of TLESRs. It has been suggested that patients with GERD have an increased frequency of TLESR compared to healthy subjects (32), but others reported that the frequency of TLESR is not significantly different between patients with GERD and controls (33). After a mixed meal the rate of TLESR showed a trend towards higher values (not significant) in patients with GERD compared with healthy subjects (27).

Mechanisms of reflux

Absence of LES pressure or very low residual LES pressure is a prerequisite for the occurrence of gastroesophageal reflux. During intervals of negligible LES pressure, increments of intra-abdominal pressure results in gastroesophageal reflux, but intra-abdominal increases of LES pressure alone do not increases gastroesophageal reflux (34). During TLESR gastroesophageal reflux may occur. Not only the frequency but probably the number of TLESRs associated with reflux is significantly higher in patients with GERD compared to controls (32). In patients with a hiatus hernia excess of gastroesophageal reflux is not caused by TLESR but by other mechanisms like low LES pressure, swallow associated normal LES relaxations or abdominal straining during low LES pressure (35).

In children TLESRs are the most common mechanism of reflux (10; 36; 37). Ambulatory manometry studies have confirmed results of studies with stationary manometry that TLESRs are the main mechanism of GER in healthy subjects (38; 39).
Gastroesophageal reflux of air, belches, are associated with TLESR, as is the case with acid reflux episodes (40) Belching produces a characteristic noise that is preceded by a common cavity. A common cavity phenomenon is defined as a sharp rise in esophageal pressure to the level of gastric pressure occurring within 1 sec and is accompanied by complete LES relaxation (41).

Patterns of gas and liquid reflux during TLESR were studied using intraluminal electrical impedance. Both liquid and gas reflux occurs during TLESR although acid reflux occurs as a primary event (42). Patients with GERD have more acid reflux and less nonacid reflux compared to controls. Difference in the air-liquid composition of the refluxate may contribute to higher rate of acid reflux in patients with GERD (43).

Neural regulation

A neural pathway underlying TLESRs has been suggested. The afferent pathway of the reflex arc consists of stretch sensitive vagal afferent fibers presumably located within the muscle layers of the proximal stomach. The vagal afferent fibers, activated during gastric distension, terminate in the nucleus tractus solitarius and the dorsal motor nucleus of the vagal nerve. The motor neurons located in the nucleus tractus solitarius synapse with dendrites from the motor neurons located in the dorsal motor nucleus of the vagal nerve and

the nucleus ambiguus, which in turn project to the enteric nervous system of the esophagus, LES and stomach. This circuitry enables fast vago-vagal reflexes and is presumably involved in mediating TLESR (7; 44).

In dogs, cooling of the vagal nerve decreased the number of TLESR (45). In dogs, TLESR were abolished general anesthesia (46), but another study showed that in cats anesthetized using ketamine (light anesthesia) TLESR were still present. The muscarine receptor antagonist atropine inhibited gastric distension induced TLESR compared with placebo (47) in healthy controls and in patients with GERD (48). In patients with achalasia TLESR do not occur after gastric distention supporting neural mediation of TLESR (49). Morphine decreases the number of TLESR (50).

Nitric oxide (NO) is a nonpeptide transmitter produced in the gut. The nitric oxide synthase inhibitor N^G-nitro-L-arginine-methyl esther (L-NAME) inhibited gastric distension induced TLESR in dogs (51). Nitric oxide synthesis inhibition by N^G-monomethyl-L-arginine (L-NMMA) reduced the number of TLESR triggered by meal induced or gastric balloon gastric distension in human (52; 53). The neurotransmitter nitric oxide is involved in the occurrence of LES relaxation.

Gastrointestinal peptides and transmitters

Gut peptides function as messenger molecules in the gastrointestinal tract. They may function as hormones, neuropeptides, paracrine or autocrine agents. Patients with GERD may have an altered serum hormonal profile. The hormonal abnormalities are more marked in patients with low LES pressure. It is not known whether these changes are primary or secondary (54). Gastrointestinal peptides may be involved in the regulation of lower esophageal sphincter.

Cholecystokinin-A receptors are located on vagal afferents. Cholecystokinin-33 decrease LES pressure only when infused at plasma levels comparable with those reached after ingestion of high fat meals. Cholecystokinin-33 had no effect on the frequency of TLESR (26). Cholecystokinin-8 increases the number of TLESR (51; 55). Endogenous cholecystokinin, stimulated by cholestyramine, increased the frequency of TLESR (56). Devazepide, a cholecystokinin-8 receptor antagonist, reduces the number of TLESR after gastric distension in dog. Loxiglumide another cholecystokinin-A receptor antagonist also inhibits TLESR (56-58).

Gastrin may increase reflux by stimulating acid secretion. Previous studies indicated that gastrin may increases LES pressure, however when gastrin is administered in doses similar to those reached after ingestion of a protein meal, no effect on LES pressure was observed or LES pressure even decreased. The effect of gastrin on TLESR had not been investigated (59-65).

Somatostatin is a polypeptide known for its inhibitory effect on the gastrointestinal tract (66-70). Somatostatin inhibits gastric acid secretion and may increase LES pressure (68; 70). The effect of somatostatin on reflux mechanisms, especially TLESR, was unknown.
The tetradecapeptide bombesin affects gastrointestinal secretion and motility. Bombesin increases LES pressure. This effect of bombesin is not influenced by atropine or somatostatin (71). The effect of bombesin on TLESR had not been investigated.

Glutamate is thought to be a major transmitter of vagal afferents. It has been suggested that glutamate may be involved in the neurocircuitry underlying TLESR. However

in healthy subjects riluzole, a glutamate release inhibitor, reduces the number of TLESR evoked by gastric distension.(72)

Serotoninergic 5-HT3 receptors are located on vagal fibers. The 5-HT3 antagonists ondansetron and granisetron, reduced the number of TLESR induced by i.v. cholecystokinin-8 in dogs (73).

Baclofen, a $GABA_B$ receptor antagonist, inhibits gastric distension induced TLESR in the ferret (74; 75) and in humans (48; 76).

In GERD patients tegaserod, a 5HT4 receptor partial agonist with prokinetic effects, decreases frequency of TLESR (77).

Esophageal body motility

Gastroesophageal acid reflux occurs predominately during TLESR and when LES pressure is very low (78). The severity of reflux esophagitis is related not only to the frequency but also to the duration of gastroesophageal reflux episodes (79; 80). Therefore another factor like esophageal body motility plays an important role in GERD. Clearance of acid refluxate from the esophagus is dependent on the efficacy of esophageal peristalsis. Previous studies using stationary manometry have shown that esophageal motility is impaired in patients with GERD. No improvement in peristaltic activity after healing of esophagitis has been observed (81; 82). It has been suggested that impaired motility in reflux esophagitis is an irreversible consequence of esophageal inflammation but others have also pointed to impaired motility as a preexistent factor in the pathogenesis of reflux. Sleep may impair esophageal acid clearance, but arousal from sleep ensures normal swallow responses to acid reflux and characteristics of peristaltic contractions are not altered by the level of consciousness (83; 84).

Management of gastroesophageal reflux disease

Acid suppression is the therapy of choice in patients with GERD. Prokinetic drugs may have additional benefit in patients with delayed gastric emptying. In patients refractory to prolonged and intensive medical therapy surgical treatment should be considered. Fundoplication of the esophagogastric junction is the most used surgical procedure. Complete (360° wrap) Nissen fundoplication or partial (180°-270° wrap) fundoplication (Toupet, Belsey Mark IV) have been performed. Fundoplication either by open or by laparoscopic route increases pressure at the esophagogastric junction to levels in the normal range (85). After fundoplication the frequency of TLESR is decreased (86-89). Postfundoplication acid reflux is diminished to values in the normal range (90). Also symptoms of reflux improve. Dysphagia is a known complication of fundoplication which may be treated with endoscopic dilatation. However some patients need surgical reintervention by changing the wrap from complete to partial fundoplication, or even undo the fundoplication. Damage of the vagal nerve may lead to symptoms of diarrhea.

Recently, a number of endoscopic techniques have been developed aimed at improving the esophagogastric junction to prevent gastroesophageal reflux. Reduction of GERD symptoms, reduction of requirement for acid suppressant medication and reduction in acid reflux has been reported in studies using delivery of radiofrequency energy (91), endoscopic deep mural implantation of polymer (92) or endoscopic plication at esophagogastric junction (93). Delivery of radiofrequency energy reduced the rate of postprandial TLESR (91). However, convincing results have been obtained in only two-thirds of patients with median follow up of 6 months. Moreover, several inconsistencies have

emerged between the efficacy of this form of treatment in improving symptoms and quality of life ad a lack of improvement of objective parameter such as lower esophageal sphincter pressure and esophageal acid exposure (94).

In conclusion, several studies have shown that TLESR play a major role in gastroesophageal reflux. Gastric distension is a potent trigger for TLESR. Inhibition of TLESR frequency is considered as a therapeutic goal in GERD patients and substances that affect TLESRs are being explored for their efficacy. Knowledge on mechanisms of reflux is relevant for control of gastroesophageal reflux especially in patients with GERD.

AIMS AND OUTLINE OF INVESTIGATION

The studies presented in this thesis have been designed to gain more insight into reflux mechanisms. Especially the role of TLESR in gastroesophageal reflux was investigated. Esophageal motility was studied under standardized conditions using esophageal manometry combined with pH-metry. Healthy subjects, patients with GERD and patients after antireflux surgery participated in these studies.

In *chapter 2* we studied LES characteristics in a large cohort of patients with GERD. LES pressure, TLESR and mechanisms of reflux were investigated before and after ingestion of a meal. A meal was used to trigger TLESR. We have investigated whether the frequency of TLESR is increased in patients with GERD compared to healthy controls. Secondly we investigated whether the frequency of TLESR is related to the severity of GERD, presence of a hiatal hernia or LES pressure.

Gastric distension is considered a potent trigger for TLESR. In *chapter 3* we explored the effect of an acute gastric distension by intragastric insufflation with air. The intragastric air will leave the stomach by belching. TLESR and mechanisms of belching were studied in patients with GERD and patients after fundoplication and results were compared with those obtained in healthy controls.

In *chapter 4* we explored the effect of a prolonged gastric distension using an intragastric bag. The intragastric bag is controlled by a barostat system to maintain constant intragastric pressures with variable intragastric bag volumes. The aim of the study was to explore the relation between gastric distension and TLESR.

We studied in *chapter 5* the effect of intravenous gastrin. Ingestion of a meal increases the frequency of TLESR and stimulates gastrin release. Supra-physiological doses of gastrin influence LES pressure. Since gastrin induces acid secretion, gastrin may also be a cause of acid reflux, however the effect of gastrin on TLESR is not known. We have investigated LES pressure and TLESR during infusion of gastrin at doses comparable with postprandial levels of gastrin. Healthy volunteers were investigated under basal, fasting conditions.

We studied in *chapter 6* the effect of intravenous somatostatin. Somatostatin has an inhibitory effect on several functions in the gastrointestinal tract. Somatostatin inhibits gastric acid secretion and may increase LES pressure. Somatostatin is therefore of potential clinical interest to reduce gastroesophageal reflux. The effects of somatostatin were investigated in healthy subjects under basal fasting conditions and after ingestion of a meal. Carbohydrate-rich, low fat meals were used to trigger TLESR.

Chapter 7 deals with the effect of bombesin on TLESR. An increased LES pressure inhibits gastroesophageal reflux. Patients with GERD may have low LES pressure. The LES pressure can be surgically increased by fundoplication. Bombesin also elevates LES pressure, but the effect of bombesin on TLESR and mechanism of reflux is not known. We studied the

effect of bombesin on LES characteristics and reflux mechanisms in patients with GERD, patients after antireflux surgery and healthy controls. Gastric distension with air was used to trigger TLESR.

Non-adrenergic non-cholinergic (NANC) nerves mediate inhibitory responses such as relaxation of the LES after swallowing. Nitric oxide (NO) is known as an inhibitory neurotransmitter of NANC nerves. In *chapter 8* we investigated the influence of l-arginine, a NO precursor, on TLESR in healthy subjects. Carbohydrate-rich, high fat meals were used to trigger TLESR.

The sphinctometer is a solid state manometry system designed for sphincter manometry under ambulatory conditions. In *chapter 9* we have compared the sphinctometer with the water perfused sleeve device in healthy subjects. LES characteristics especially TLESR and mechanisms of reflux were measured simultaneously with sleeve and sphinctometer.

In *chapter 10* we performed prolonged ambulatory LES pressure measurements using a sphinctometer. LES pressure, TLESR frequency and mechanisms of reflux were studied during upright and supine episodes in twenty-four hour registrations. Patients with GERD and healthy controls participated in the study.

Esophageal body motility is another factor involved in gastroesophageal reflux, in addition to TLESR and LES pressure. The role of esophageal motility on the clearance of acid reflux was studied in *chapter 11*. Esophageal clearance of acid is performed by peristaltic esophageal contractions. Clearance of acid may be dependent on characteristics of esophageal contractions and the efficacy of peristalsis. Esophageal motility was analyzed during episodes of gastroesophageal reflux. Ambulatory esophageal pH and manometry was performed in healthy subjects and patients with GERD.

The effect of surgical therapy on patients with GERD was studied in *chapter 12*. In a prospective study the effects of laparoscopic Nissen fundoplication on TLESR and LES pressure and reflux mechanisms were investigated.

Finally, the results of the various studies presented in this thesis are summarized and discussed.

REFERENCES

1. Dent J, El Serag HB, Wallander MA, Johansson S. Epidemiology of gastro-oesophageal reflux disease: a systematic review. Gut 2005;54:710-717.
2. Kahrilas PJ, Lin S, Chen J, Manka M. The effect of hiatus hernia on gastro-oesophageal junction pressure. Gut 1999;44:476-482.
3. Masclee AAM, de Best AC, de Graaf R, Cluysenaer OJ, Jansen JB. Ambulatory 24-hour pH-metry in the diagnosis of gastroesophageal reflux disease. Determination of criteria and relation to endoscopy. Scand J Gastroenterol 1990;25:225-230.
4. Kahrilas PJ, Quigley EM. Clinical esophageal pH recording: a technical review for practice guideline development. Gastroenterology 1996;110:1982-1996.
5. Coenraad M, Masclee AAM, Straathof JWA, Ganesh S, Griffioen G, Lamers CBHW. Is Barrett's esophagus characterized by more pronounced acid reflux than severe esophagitis? Am J Gastroenterol 1998;93:1068-1072.
6. Mittal RK, Lange RC, McCallum RW. Identification and mechanism of delayed esophageal acid clearance in subjects with hiatus hernia. Gastroenterology 1987;92:130-135.
7. Mittal RK, Holloway RH, Penagini R, Blackshaw LA, Dent J. Transient lower esophageal sphincter relaxation. Gastroenterology 1995;109:601-610.
8. Dent J. A new technique for continuous sphincter pressure measurement. Gastroenterology 1976;71:263-267.
9. Omari TI, Miki K, Davidson G, Fraser R, Haslam R, Goldsworthy W, Bakewell M, Dent J. Characterisation of relaxation of the lower oesophageal sphincter in healthy premature infants. Gut 1997;40:370-375.
10. Omari TI, Barnett CP, Benninga MA, Lontis R, Goodchild L, Haslam RR, Dent J, Davidson GP. Mechanisms of gastro-oesophageal reflux in preterm and term infants with reflux disease. Gut 2002;51:475-479.
11. van Herwaarden MA, Samsom M, Akkermans LM, Smout AJ. Prolonged recording of oesophageal and lower oesophageal sphincter pressure using a portable water-perfused manometric system. Neurogastroenterol Motil 2001;13:111-119.
12. Trudgill NJ, Riley SA. Monitoring the lower oesophageal sphincter: sphinctometer or sleeve? Neurogastroenterol Motil 1999;11:173-178.
13. Pehlivanov N, Liu J, Arora T, Yamamoto Y, Mittal RK. Lower esophageal sphincter monitoring with sphinctometer: in vitro and in vivo studies. Am J Physiol 1999;277:G577-G584.
14. Holloway RH, Penagini R, Ireland AC. Criteria for objective definition of transient lower esophageal sphincter relaxation. Am J Physiol 1995;268:G128-G133.
15. Van Herwaarden M, Samsom M, Wolf C, Leong IS, Smout AJ. Computer analysis of prolonged lower oesophageal sphincter pressure recordings. Neurogastroenterol Motil 2001;13:37-44.
16. Weusten BL, Akkermans LM, Vanberge-Henegouwen GP, Smout AJ. Dynamic characteristic of gastro-oesophageal reflux in ambulatory patients with gastro-oesophageal reflux disease and normal control subjects. Scand J Gastroenterol 1995;30:731-737.
17. Mittal RK, Stewart WR, Schirmer BD. Effect of a catheter in the pharynx on the frequency of transient lower esophageal sphincter relaxations. Gastroenterology 1992;103:1236-1240.
18. Mittal RK, Chiareli C, Liu J, Shaker R. Characteristics of lower esophageal sphincter relaxation induced by pharyngeal stimulation with minute amounts of water. Gastroenterology 1996;111:378-384.
19. Noordzij JP, Mittal RK, Arora T, Pehlivanov N, Liu J, Reibel JF, Levine PA. The effect of mechanoreceptor stimulation of the laryngopharynx on the oesophago-gastric junction. Neurogastroenterol Motil 2000;12:353-359.
20. Ireland AC, Dent J, Holloway RH. Preservation of postural control of transient lower oesophageal sphincter relaxations in patients with reflux oesophagitis. Gut 1999;44:313-316.

21. Ireland A, Dent J, Holloway RH. The role of head position in the postural control of transient lower oesophageal sphincter relaxations and belching. Gullet 1992;2:81-84.
22. Freidin N, Mittal RK, McCallum RW. Does body posture affect the incidence and mechanism of gastro- oesophageal reflux? Gut 1991;32:133-136.
23. Freidin N, Fisher MJ, Taylor W, Boyd D, Surratt P, McCallum RW, Mittal RK. Sleep and nocturnal acid reflux in normal subjects and patients with reflux oesophagitis. Gut 1991;32:1275-1279.
24. Holloway RH, Kocyan P, Dent J. Provocation of transient lower esophageal sphincter relaxations by meals in patients with symptomatic gastroesophageal reflux. Dig Dis Sci 1991;36:1034-1039.
25. Penagini R, Bartesaghi B, Bianchi PA. Effect of cold stress on postprandial lower esophageal sphincter competence and gastroesophageal reflux in healthy subjects. Dig Dis Sci 1992;37:1200-1205.
26. Ledeboer M, Masclee AAM, Batstra MR, Jansen JB, Lamers CBHW. Effect of cholecystokinin on lower oesophageal sphincter pressure and transient lower oesophageal sphincter relaxations in humans. Gut 1995;36:39-44.
27. Penagini R, Mangano M, Bianchi PA. Effect of increasing the fat content but not the energy load of a meal on gastro-oesophageal reflux and lower oesophageal sphincter motor function [see comments]. Gut 1998;42:330-333.
28. Holloway RH, Hongo M, Berger K, McCallum RW. Gastric distention: a mechanism for postprandial gastroesophageal reflux. Gastroenterology 1985;89:779-784.
29. Franzi SJ, Martin CJ, Cox MR, Dent J. Response of canine lower esophageal sphincter to gastric distension. Am J Physiol 1990;259:G380-G385.
30. Stakeberg J, Lehmann A. Influence of different intragastric stimuli on triggering of transient lower oesophageal sphincter relaxation in the dog. Neurogastroenterol Motil 1999;11:125-132.
31. Dent J, Dodds WJ, Friedman RH, Sekiguchi T, Hogan WJ, Arndorfer RC, Petrie DJ. Mechanism of gastroesophageal reflux in recumbent asymptomatic human subjects. J Clin Invest 1980;65:256-267.
32. Dodds WJ, Dent J, Hogan WJ, Helm JF, Hauser R, Patel GK, Egide MS. Mechanisms of gastroesophageal reflux in patients with reflux esophagitis. N Engl J Med 1982;307:1547-1552.
33. Mittal RK, McCallum RW. Characteristics and frequency of transient relaxations of the lower esophageal sphincter in patients with reflux esophagitis. Gastroenterology 1988;95:593-599.
34. Dent J, Dodds WJ, Hogan WJ, Toouli J. Factors that influence induction of gastroesophageal reflux in normal human subjects. Dig Dis Sci 1988;33:270-275.
35. van Herwaarden MA, Samsom M, Smout AJ. Excess gastroesophageal reflux in patients with hiatus hernia is caused by mechanisms other than transient LES relaxations. Gastroenterology 2000;119:1439-1446.
36. Cucchiara S, Bortolotti M, Minella R, Auricchio S. Fasting and postprandial mechanisms of gastroesophageal reflux in children with gastroesophageal reflux disease. Dig Dis Sci 1993;38:86-92.
37. Cucchiara S, Minella R, Campanozzi A, Salvia G, Borrelli O, Ciccimarra E, Emiliano M. Effects of omeprazole on mechanisms of gastroesophageal reflux in childhood. Dig Dis Sci 1997;42:293-299.
38. Penagini R, Schoeman MN, Dent J, Tippett MD, Holloway RH. Motor events underlying gastro-oesophageal reflux in ambulant patients with reflux oesophagitis. Neurogastroenterol Motil 1996;8:131-141.
39. Schoeman MN, Tippett MD, Akkermans LM, Dent J, Holloway RH. Mechanisms of gastroesophageal reflux in ambulant healthy human subjects. Gastroenterology 1995;108:83-91.
40. Wyman JB, Dent J, Heddle R, Dodds WJ, Toouli J, Downton J. Control of belching by the lower oesophageal sphincter. Gut 1990;31:639-646.

41. Patrikios J, Martin CJ, Dent J. Relationship of transient lower esophageal sphincter relaxation to postprandial gastroesophageal reflux and belching in dogs. Gastroenterology 1986;90:545-551.
42. Sifrim D, Silny J, Holloway RH, Janssens JJ. Patterns of gas and liquid reflux during transient lower oesophageal sphincter relaxation: a study using intraluminal electrical impedance. Gut 1999;44:47-54.
43. Sifrim D, Holloway R, Silny J, Tack J, Lerut A, Janssens J. Composition of the postprandial refluxate in patients with gastroesophageal reflux disease. Am J Gastroenterol 2001;96:647-655.
44. Mittal RK, Balaban DH. The esophagogastric junction. N Engl J Med 1997;336:924-932.
45. Martin CJ, Patrikios J, Dent J. Abolition of gas reflux and transient lower esophageal sphincter relaxation by vagal blockade in the dog. Gastroenterology 1986;91:890-896.
46. Cox MR, Martin CJ, Dent J, Westmore M. Effect of general anaesthesia on transient lower oesophageal sphincter relaxations in the dog. Aust N Z J Surg 1988;58:825-830.
47. Mittal RK, Chiareli C, Liu J, Holloway RH, Dixon W, Jr. Atropine inhibits gastric distension and pharyngeal receptor mediated lower oesophageal sphincter relaxation. Gut 1997;41:285-290.
48. Lidums I, Checklin H, Mittal RK, Holloway RH. Effect of atropine on gastro-oesophageal reflux and transient lower oesophageal sphincter relaxations in patiens with gastro-oesophageal reflux disease. Gut 1999;43:12-16.
49. Holloway RH, Wyman JB, Dent J. Failure of transient lower oesophageal sphincter relaxation in response to gastric distension in patients with achalasia: evidence for neural mediation of transient lower oesophageal sphincter relaxations. Gut 1989;30:762-767.
50. Penagini R, Bianchi PA. Effect of morphine on gastroesophageal reflux and transient lower esophageal sphincter relaxation. Gastroenterology 1997;113:409-414.
51. Boulant J, Fioramonti J, Dapoigny M, Bommelaer G, Bueno L. Cholecystokinin and nitric oxide in transient lower esophageal sphincter relaxation to gastric distention in dogs. Gastroenterology 1994;107:1059-1066.
52. Hirsch DP, Tiel-Van Buul MM, Tytgat GN, Boeckxstaens GE. Effect of L-NMMA on postprandial transient lower esophageal sphincter relaxations in healthy volunteers. Dig Dis Sci 2000;45:2069-2075.
53. Hirsch DP, Holloway RH, Tytgat GN, Boeckxstaens GE. Involvement of nitric oxide in human transient lower esophageal sphincter relaxations and esophageal primary peristalsis. Gastroenterology 1998;115:1374-1380.
54. Perdikis G, Wilson P, Hinder RA, Redmond EJ, Wetscher GJ, Saeki S, Adrian TE. Gastroesophageal reflux disease is associated with enteric hormone abnormalities. Am J Surg 1994;167:186-91.
55. Rouzade ML, Fioramonti J, Bueno L. Role of 5-HT3 receptors in the control by cholecystokinin of transient relaxations of the inferior esophageal sphincter in dogs. Gastroenterol Clin Biol 1996;20:575-580.
56. Clave P, Gonzalez A, Moreno A, Lopez R, Farre A, Cusso X, D'mato M, Azpiroz F, Lluis F. Endogenous cholecystokinin enhances postprandial gastroesophageal reflux in humans through extrasphincteric receptors. Gastroenterology 1998;115:597-604.
57. Boulant J, Mathieu S, D'Amato M, Abergel A, Dapoigny M, Bommelaer G. Cholecystokinin in transient lower oesophageal sphincter relaxation due to gastric distension in humans. Gut 1997;40:575-581.
58. Boeckxstaens GE, Hirsch DP, Fakhry N, Holloway RH, D'Amato M, Tytgat GNJ. Involvement of cholecystokinin-A receptors in transient lower esophageal sphincter relaxations triggered by gastric distension. Am J Gastroenterol 1998;93:1823-1828.
59. Castell DO, Harris LD. Hormonal control of gastroesophageal-sphincter strength. N Engl J Med 1970;282:886-889.
60. Walker CO, Frank SA, Manton J, Fordtran JS. Effect of continuous infusion of pentagastrin on lower esophageal sphincter pressure and gastric acid secretion in normal subjects. J Clin Invest

1975;56:218-225.
61. Freeland GR, Higgs RH, Castell DO, McGuigan JE. Lower esophageal sphincter and gastric acid response to intravenous infusions of synthetic human gastrin I heptadecapeptide. Gastroenterology 1976;71:570-574.
62. Henderson JM, Lidgard G, Osborne DH, Carter DC, Heading RC. Lower oesophageal sphincter response to gastrin--pharmacological or physiological? Gut 1978;19:99-102.
63. Jensen DM, McCallum RW, Corazziari E, Elashoff J, Walsh JH. Human lower esophageal sphincter responses to synthetic human gastrins 34 (G-34) and 17 (G-17). Gastroenterology 1980;79:431-438.
64. Allescher HD, Stoschus B, Wunsch E, Schusdziarra V, Classen M. Effect of human gastrin-17 with and without acid suppression on human esophageal motility. Z Gastroenterol 1995;33:385-391.
65. Lepsien G, Dietrich K. Peptone stimulation of the lower esophageal sphincter in patients with reflux disease. Z Gastroenterol 1988;26:209-216.
66. Peeters TL, Janssens J, Vantrappen GR. Somatostatin and the interdigestive migrating motor complex in man. Regul Pept 1983;5:209-217.
67. Krejs GJ. Physiological role of somatostatin in the digestive tract: gastric acid secretion, intestinal absorption, and motility. Scand J Gastroenterol Suppl 1986;119:47-53.
68. Shulkes A. Somatostatin: physiology and clinical applications. Baillieres Clin Endocrinol Metab 1994;8:215-236.
69. Lieverse RJ, Jansen JB, Jebbink MC, Masclee AAM, Rovati LC, Lamers CBHW. Effects of somatostatin and loxiglumide on gallbladder motility. Eur J Clin Pharmacol 1995;47:489-492.
70. Gunshefski LA, Rifley WJ, Slattery DW, Schifini JJ, Hartsuck M, Little AG. Somatostatin stimulation of the normal esophagus. Am J Surg 1992;163:59-62.
71. Masclee AA, Lam WF, Lamers CB. Effect of bombesin on esophageal motility in humans. Dis Esophagus 1999;12:54-59.
72. Hirsch DP, Tytgat GN, Boeckxstaens GE. Is glutamate involved in transient lower esophageal sphincter relaxations? Dig Dis Sci 2002;47:661-666.
73. Rouzade ML, Fioramonti J, Bueno L. Role of 5-HT3 receptors in the control by cholecystokinin of transient relaxations of the inferior esophageal sphincter in dogs. Gastroenterol Clin Biol 1996;20:575-580.
74. Blackshaw LA, Staunton E, Lehmann A, Dent J. Inhibition of transient LES relaxations and reflux in ferrets by GABA receptor agonists. Am J Physiol 1999;277:G867-G874.
75. Staunton E, Smid SD, Dent J, Blackshaw LA. Triggering of transient LES relaxations in ferrets: role of sympathetic pathways and effects of baclofen. Am J Physiol Gastrointest Liver Physiol 2000;279:G157-G162.
76. Lidums I, Lehmann A, Checklin H, Dent J, Holloway RH. Control of transient lower esophageal sphincter relaxations and reflux by the GABA(B) agonist baclofen in normal subjects. Gastroenterology 2000;118:7-13.
77. Kahrilas PJ, Quigley EM, Castell DO, Spechler SJ. The effects of tegaserod (HTF 919) on oesophageal acid exposure in gastro-oesophageal reflux disease. Aliment Pharmacol Ther 2000;14:1503-1509.
78. Dent J, Holloway RH, Toouli J, Dodds WJ. Mechanisms of lower oesophageal sphincter incompetence in patients with symptomatic gastrooesophageal reflux. Gut 1988;29:1020-1028.
79. Timmer R, Breumelhof R, Nadorp JH, Smout AJ. Oesophageal motor response to reflux is not impaired in reflux oesophagitis. Gut 1993;34:317-320.
80. Kasapidis P, Xynos E, Mantides A, Chrysos E, Demonakou M, Nikolopoulos N, Vassilakis JS. Differences in manometry and 24-H ambulatory pH-metry between patients with and without endoscopic or histological esophagitis in gastroesophageal reflux disease. Am J Gastroenterol 1993;88:1893-1899.

81. McDougall NI, Mooney RB, Ferguson WR, Collins JS, McFarland RJ, Love AH. The effect of healing oesophagitis on oesophageal motor function as determined by oesophageal scintigraphy and ambulatory oesophageal motility/pH monitoring. Aliment Pharmacol Ther 1998;12:899-907.
82. Timmer R, Breumelhof R, Nadorp JH, Smout AJ. Oesophageal motility and gastro-oesophageal reflux before and after healing of reflux oesophagitis. A study using 24 hour ambulatory pH and pressure monitoring. Gut 1994;35:1519-1522.
83. Orr WC, Robinson MG, Johnson LF. Acid clearance during sleep in the pathogenesis of reflux esophagitis. Dig Dis Sci 1981;26:423-427.
84. Orr WC, Johnson LF, Robinson MG. Effect of sleep on swallowing, esophageal peristalsis, and acid clearance. Gastroenterology 1984;86:814-819.
85. Mathew G, Watson DI, Myers JC, Holloway RH, Jamieson GG. Oesophageal motility before and after laparoscopic Nissen fundoplication. Br J Surg 1997;84:1465-1469.
86. Ireland AC, Holloway RH, Toouli J, Dent J. Mechanisms underlying the antireflux action of fundoplication. Gut 1993;34:303-308.
87. Masclee AAM, Horbach JM, Ledeboer M, Lamers CBHW. Prospective study of the effect of the Belsey Mark-IV fundoplication on reflux mechanisms. Scand J Gastroenterol 1998;33:905-910.
88. Lindeboom MA, Ringers J, Straathof JW, van Rijn PJ, Neijenhuis P, Masclee AA. Effect of laparoscopic partial fundoplication on reflux mechanisms. Am J Gastroenterol 2003;98:29-34.
89. Scheffer RC, Tatum RP, Shi G, Akkermans LM, Joehl RJ, Kahrilas PJ. Reduced tLESR elicitation in response to gastric distension in fundoplication patients. Am J Physiol Gastrointest Liver Physiol 2003;284:G815-G820.
90. Horbach JM, Cnossen MH, Jansen JB, Lamers CBHW, Zwinderman AH, Terpstra JL, Gooszen HG. A prospective study of effects of Belsey MK IV antireflux surgery on endoscopic esophagitis, lower esophageal sphincter pressure, and 24-hr pH measurements. Relation to symptom improvement. Dig Dis Sci 1994;39:385-392.
91. Tam WC, Schoeman MN, Zhang Q, Dent J, Rigda R, Utley D, Holloway RH. Delivery of radiofrequency energy to the lower oesophageal sphincter and gastric cardia inhibits transient lower oesophageal sphincter relaxations and gastro-oesophageal reflux in patients with reflux disease. Gut 2003;52:479-485.
92. Johnson DA, Ganz R, Aisenberg J, Cohen LB, Deviere J, Foley TR, Haber GB, Peters JH, Lehman GA. Endoscopic, deep mural implantation of enteryx for the treatment of GERD: 6-month follow-up of a multicenter trial. Am J Gastroenterol 2003;98:250-258.
93. Mahmood Z, McMahon BP, Arfin Q, Byrne PJ, Reynolds JV, Murphy EM, Weir DG. Endocinch therapy for gastro-oesophageal reflux disease: a one year prospective follow up. Gut 2003;52:34-39.
94. Annese V, Caletti G, Cipolletta L, Costamagna G, D'Onofrio V, Leandro G, Koch M, Pace F, Penagini R, Repici A, Ricci E, Vigneri S, Zaninotto G. Endoscopic treatment of gastroesophageal reflux disease. Endoscopy 2005;37:470-478.

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TRANSIENT LOWER ESOPHAGEAL SPHINCTER RELAXATIONS AS REFLUX MECHANISM IN GASTROESOPHAGEAL REFLUX DISEASE: A QUANTITATIVE ANALYSIS.

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Submitted for publication

ABSTRACT

Background: Transient lower esophageal sphincter relaxations (TLESRs) are considered the major mechanism for gastroesophageal reflux but data from large scale studies on TLESRs and other reflux mechanisms in gastroesophageal reflux disease are still lacking.
Methods: Between 1995 and 2002 114 patients with documented GERD and 30 healthy controls underwent combined esophageal pH metry and sleeve manometry one hour before and 3 hours after ingestion of a mixed liquid meal (2520 kJ). TLESR frequency, gastroesophageal reflux and mechanisms of reflux were scored.
Results: Fasting TLESR frequency was not significantly different between GERD patients and controls 3.3 ± 0.3 vs 2.8 ± 0.4 per hour. Postprandial TLESR frequency was significantly ($p<0.05$) reduced in GERD patients vs. controls; first postprandial hour: 4.6 ± 0.3 vs. 6.1 ± 0.6 per hour. The percentage of TLESRs associated with reflux was significantly higher in GERD patients vs. controls: 42 ± 3 vs. 16 ± 3% ($p<0.001$). In GERD patients TLESRs accounted for 49% of reflux episodes versus 82% in controls ($p<0.05$). Neither the presence of a hiatal hernia nor degree of esophagitis affected the mechanism of reflux. However, reflux mechanisms were significantly related to fasting LES pressure.
Conclusions: In GERD patients the frequency of TLESRs is not increased over controls and postprandially the TLESR frequency is even significantly decreased. In GERD patients significantly more TLESRs are associated with acid reflux. Subgroups of GERD patients exist according to reflux type. Fasting LES pressure is an important predictor of reflux type and reflux mechanisms.

INTRODUCTION

Gastroesophageal reflux (GER) has a multifactorial origin. The esophagogastric junction functions as a barrier that only allows reflux of gastric content when lower esophageal sphincter (LES) pressure is low or during relaxations of the LES (1-3). The duration of a gastroesophageal reflux episode is dependent on esophageal factors that contribute to luminal clearance mechanisms such as peristalsis and buffering by saliva. Transient LES relaxations (TLESR) are spontaneous relaxations of the esophagogastric junction in the absence of a preceding swallowing event (3). TLESRs have been well recognized as an important mechanism of gastroesophageal reflux. Several reports have stated that in patients with gastroesophageal reflux disease (GERD) the number of TLESRs is significantly increased compared to healthy controls (2, 4-6). In healthy subjects TLESRs account for 80-100% of all reflux episodes but in patient with GERD a much smaller percentage of reflux episodes results from TLESRs (1-7). In GERD patients TLESR account only for about 50% of reflux episodes (1-9). The contribution of TLESRs to reflux in GERD patients is therefore less prominent as previously has been suggested. Several reports on TLESRs in reflux disease can be criticized either because of the small number of patients and controls included, or because different protocols to provoke TLESRs have been employed or because investigators did not take into account factors as the presence of a hiatal hernia or severity of reflux disease.

The present study was undertaken to expand our knowledge on the role of TLESRs as reflux mechanism in reflux disease and to correlate TLESRs and reflux mechanisms to LES

pressure, grade of esophagitis and presence of hiatus hernia. A large cohort of GERD patients was included. Of these patients data from endoscopy and 24 hour pH metry were available. TLESRs were evaluated according to a strict protocol and for comparison a large group of healthy controls was included.

METHODS

Subjects

A group of 114 patients with documented gastroesophageal reflux disease (55 male, 59 female; mean age 46 yr, range 21-73 yr) participated in the study. Between 1995 and 2002 reflux patients visiting the Department of Gastroenterology-Hepatology of the Leiden University Medical Center were asked to participate. All patients had symptoms of gastroesophageal reflux such as heartburn, regurgitation, retrosternal pain or dysphagia. GERD had been documented previously in all patients either by endoscopy showing erosive esophagitis and/or by ambulatory 24-hour pH monitoring demonstrating an increased esophageal exposure time to acid gastric content. As controls, 30 healthy volunteers were studied (11 male, 19 female; mean age 25 yr, range 19-54 yr). None of the controls had a history of gastro-intestinal disease or surgery or was on chronic medication. Informed consent was obtained from each individual and the protocol had been approved by the ethical committee of the Leiden University Medical Center.

Endoscopy data

Reports of previously performed endoscopies were reviewed. The severity of esophagitis had been graded endoscopically according to the criteria of Savary and Miller (10). In case multiple endoscopies had been performed the highest esophagitis score was taken for further analysis. Endoscopy in patients with GERD showed no esophagitis (endoscopy negative / grade 0) in 30 patients, mild esophagitis (grade I) in 29 patients, moderate esophagitis (grade II) in 40 patients, and severe esophagitis (grade III) in 15 patients.

24 hour pH metry data

Reports of previously performed 24 hour pH metry were reviewed. Twenty-four hour ambulatory intra-esophageal pH metry had been performed with the pH electrode positioned 5 cm above the LES. A portable recorder (μdigitrapper, Medtronic, Denmark) and a glass electrode with internal reference electrode (Ingold LOT 440 continue glassreference electrode; Ingold Messtechnik AG, Urdorf, Germany) were used. As reflux parameter we used the percentage of time with pH <4 for total recording time. Pathological reflux was defined as ≥4.0% of total time with esophageal pH below 4 (11).

Manometric and pH technique

The manometry catheter consisted of a multilumen silicone tube (outer diameter 5.0 mm) with seven side holes located at 29, 23, 18, 13, 8, 3 and -4 cm from the mid of the 6 cm long sleeve sensor (Dentsleeve Pty Ltd, Belair, South Australia). The catheter was continuously perfused with gas free distilled water by a low compliance pneumohydraulic capillary infusion system at a rate of 0.5 ml/min. The external pressures transducers (Medex

Inc., Ohio, U.S.A.) were connected via an analogue/digital converter (PC Polygraph HR, Medtronic, Denmark) to a personal computer system. The data were displayed continuously on a monitor and stored on the personal computer system (Polygram Upper GI 6.30, Gastrosoft Inc., Medtronic, Denmark).

The manometry catheter was introduced through the nose into the esophagus and positioned so that the sleeve sensor straddled the LES. The proximal side hole was positioned in the pharynx and was used for identification of swallow signals. The middle side holes registered esophageal body motility. The distal side hole was used as reference point for intragastric pressure. A glass pH electrode (Ingold, Urdorf, Germany) was passed through the nose and positioned 5 cm above the upper margin of the LES. The pH electrode had been calibrated at pH 4.0 and pH 7.0.

Study protocol

The experiment was started at 9.00 a.m. after an overnight fast. The subjects were studied in the upright position, sitting in a comfortable chair. They were not allowed to doze because of the effect of sleep on TLESR. The manometry and pH catheter were introduced into the esophagus and positioned as described above. Esophageal pH and motility were registered simultaneously during a 30 min fasting period and for 180 min after a standard breakfast consisting of 400 ml Nutridrink (Numico, Zoetermeer, The Netherlands) (20 g protein, 26 g fat and 72 g carbohydrates, 2520 kJ). Subjects were asked to consume the meal within 10 min.

Lower esophageal sphincter data analysis

Lower esophageal sphincter tracings were analyzed for LES resting pressure and LES relaxations (LESR). LES pressure was defined as mean end-expiratory LES pressure relative above intragastric pressure over a 2 min period. LESR's are divided in swallow induced LESR's and spontaneous LESR's. Swallow induced LESR are preceded by active swallows starting with a pharyngeal contraction. Residual LES pressure after wet swallows was defined as end-expiratory nadir LES pressure above intragastric pressure. Spontaneous LESR's, better known as transient LES relaxations (TLESRs) are divided in non-swallow related TLESRs, and swallow related TLESR. Spontaneous, non swallow related TLESR are defined as decreases in LES pressure of ≥ 5 mmHg with a rate of ≥ 1 mmHg/sec, within 10 sec reaching a pressure of ≤ 2 mmHg above intragastric pressure. No swallow signal occurs in the interval from 4 sec before to 2 sec after onset of LESR. Swallow related TLESRs are defined as spontaneous TLESRs, irrespective of the timing of LESR to swallowing when the duration of LESR is at least 10 sec (12).

pH analysis

Gastroesophageal reflux episodes are defined as a sudden fall (< 10 sec) of pH below 4.0 with a duration of at least 4 sec. The number and duration of reflux episodes were counted.

The mechanisms of each reflux episode were scored using the following criteria. Gastroesophageal reflux occurred during:

(1) TLESR (spontaneous LESR meeting the earlier mentioned criteria; swallow related LESR with the duration of LESR ≥ 10 sec).

(2) Swallow induced LESR (primary peristalsis or failed primary peristalsis with the duration of LESR ≤ 10 sec or multiple swallowing).
(3) LES pressure drift (a gradual loss of basal LES pressure).
(4) Absent LES pressure (less than 2 mmHg above intragastric pressure).
(5) Abdominal strain (an increase in abdominal pressure).

Statistical analysis

Data are expressed as mean values ± SEM. Data were analyzed for statistical significance using Chi-square test and analysis of variance (ANOVA). When this indicated a probability of less than 0.05 for the null hypothesis, Student-Newman-Keuls analyses were performed to determine which values between or within the experiments differed significantly. A p value of <0.05 was considered significant for all analyses. Additional analysis were performed to evaluate the effect on reflux characteristics and TLESRs of:
Non-erosive vs. erosive esophagitis and degree of esophagitis (Savary-Miller);
Presence/absence of a sliding hiatus hernia;
Basal LES pressure value in three subgroups: LESP < 10mmHg (low pressure), LESP between 10-20 mmHg (intermediate pressure) and LESP > 20 mmHg (normal pressure).

RESULTS

Patients: Age and reflux

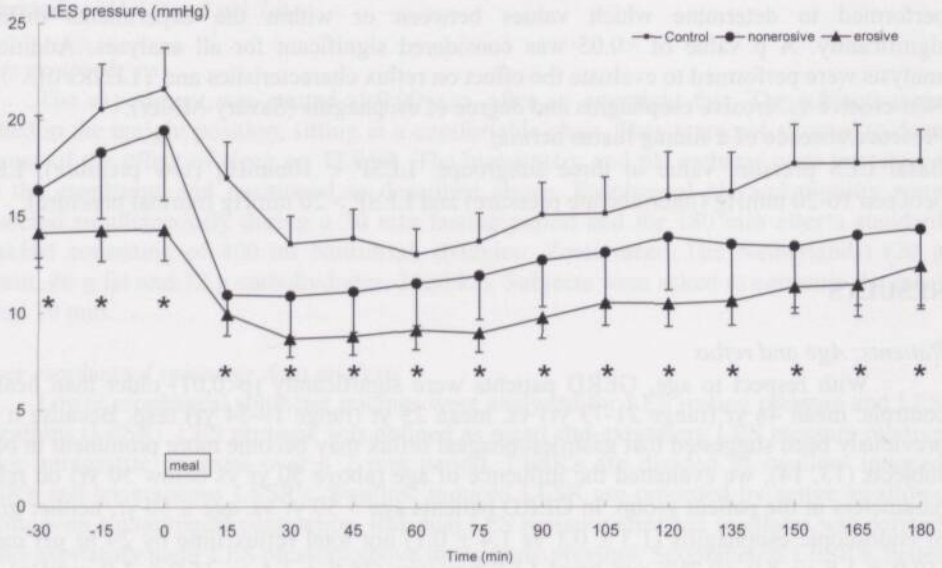
With respect to age, GERD patients were significantly (p<0.01) older than healthy controls: mean 46 yr (range 21-73 yr) vs. mean 25 yr (range 19-54 yr) resp. Because it has previously been suggested that gastroesophageal reflux may become more prominent in older subjects (13, 14), we evaluated the influence of age (above 50 yr vs below 50 yr) on reflux parameters in the patient group. In GERD patients age > 50 yr vs. age ≤ 50 yr, neither grade of endoscopic esophagitis (1.3 ± 0.1 vs 1.4 ± 0.1) nor total reflux time by 24 hr pH metry (10.0 ± 1.8 vs 8.9 ±0.7%) nor basal LES pressure (16.8 ± 1.5 vs 15.0 ± 1.0 mmHg) nor postprandial TLESR frequency (4.3 ± 0.4 vs 4.5 ± 0.3 per hour) were significantly different. Therefore differences in reflux characteristics between GERD patients and controls were considered not to be influenced by age.

Lower esophageal sphincter pressure

LES pressure in the basal period at time 0 min was significantly (p<0.01) lower in patients with GERD (16±1 mmHg) compared to healthy controls (22±2 mmHg). After ingestion of a meal LES pressure decreased significantly (p<0.001) compared to basal both in patients with GERD and in controls (Figure 1). Postprandial LES pressure was significantly (p<0.01) lower in patients with GERD compared to controls from meal ingestion until time 180 min. When reflux patients were separated into those with erosive and non-erosive reflux disease, LES pressure was not significantly different between endoscopy negative (non-erosive) GERD patients and controls: basal 19 ± 2 vs 22 ±2 mmHg and postprandially at 30 min: 11 ± 2 vs 13 ± 1 mmHg. However, the group of patients with erosive reflux disease had significantly lower basal and postprandial LES pressure compared to controls: basal 14 ± 1 vs 22 ± 2 mmHg (p<0.01) and postprandially at 30 min: 9 ± 1 vs 13 ± 1 mmHg (p<0.01). When

the patients with erosive esophagitis were further divided in subgroups based on grade of esophagitis, no significant differences in fasting or postprandial LES pressure were observed between patients with grade I, II and III esophagitis.

Figure 1. *Basal and postprandial LES pressure (mean ± SEM) in patients with erosive and non erosive reflux disease and in healthy controls. Asterisks denote significant differences between patients with erosive reflux disease vs. controls (*p< 0.01).*



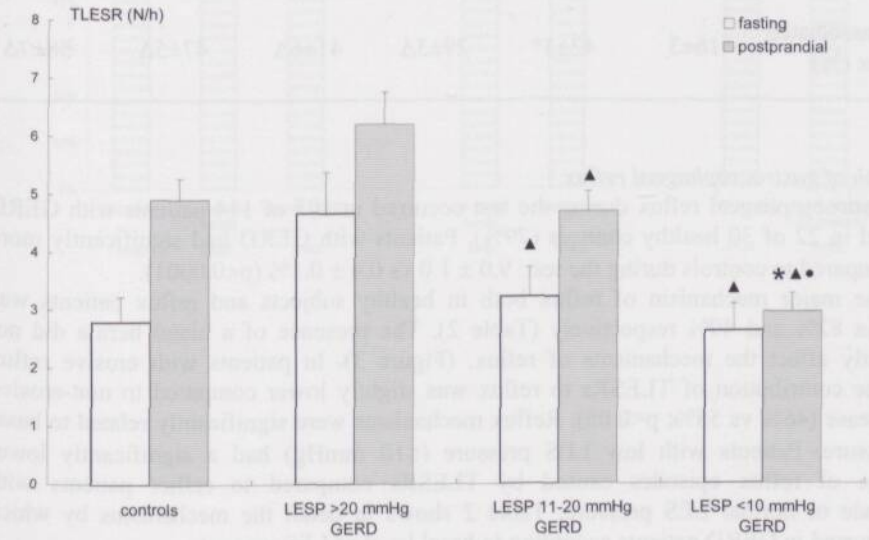
Transient lower esophageal sphincter relaxations

The frequency of TLESR in the fasting state did not significantly differ between GERD patients and controls: 3.3 ± 0.3 vs 2.8 ± 0.4 per hour. After ingestion of the meal the frequency of TLESRs increased in all groups (Table 1). For the subgroups, only in patients with grade I esophagitis was the rise in TLESR frequency over fasting statistically significant ($p<0.05$). In GERD patients postprandial TLESR frequency was significantly ($p<0.05$) lower compared to controls in the first postprandial hour. Concerning the reflux subgroups, only in patients with grade III was the difference with controls in the first postprandial hour significant ($p<0.05$). In the second and third postprandial hour no difference in TLESR frequency between patients and controls was found.

When reflux patients were divided in subgroups based on basal LES pressure, the basal TLESR frequency in GERD patients with normal LES pressure (>20 mmHg), intermediate LES pressure (10-20 mmHg) and low LES pressure (<10 mmHg) was not significantly different versus controls (Figure 2). In the intermediate and low LESP group,

basal TLESR frequency was significantly ($p<0.05$) lower compared to reflux patients with normal LESP. In the postprandial state GERD patients with low LES pressure had significantly less TLESRs compared to controls ($p<0.001$) and to GERD patients with intermediate and high LES pressure ($p<0.05$). The postprandial TLESR frequency correlated significantly ($r=0.39$; $p<0.001$) with basal LES pressure (low, intermediate, normal LES group).

Figure 2. *Frequency of transient lower esophageal sphincter relaxations (TLESR number/hour: mean±SEM) in 30 healthy controls and 114 patients with GERD. Patients are divided according to fasting LES pressure into three groups: low LES pressure (<10 mmHg); intermediate LES pressure (10-20 mmHg) and normal LES pressure (>20 mmHg). The asterisk denotes a significant difference vs. controls (p<0.001). Triangles denote significant differences between patient subgroups versus the patient group with normal LES pressure (p<0.05). The dot denotes a significant difference between patients with low vs. intermediate LES pressure (p<0.05).*



The endoscopic grade of esophagitis correlated significantly with the percentage of TLESRs associated with acid reflux (Table 1). TLESRs were accompanied by acid reflux in 29 ± 3% to 58 ± 7% in reflux patients. In healthy controls only 16 ± 3% of the TLESRs was associated with reflux.

Table 1. *Frequency of transient lower esophageal sphincter relaxations (TLESR; number/hour; mean±SEM) under fasting conditions and for 3 subsequent hours (I, II, III) after ingestion of a meal in 114 patients with GERD and 30 healthy controls. Dots denote significant differences between postprandial and fasting (p<0.05). The asterisks denote significant (p<0.05) differences compared to control. The triangles denote significant differences versus control (p<0.05-p<0.001)*

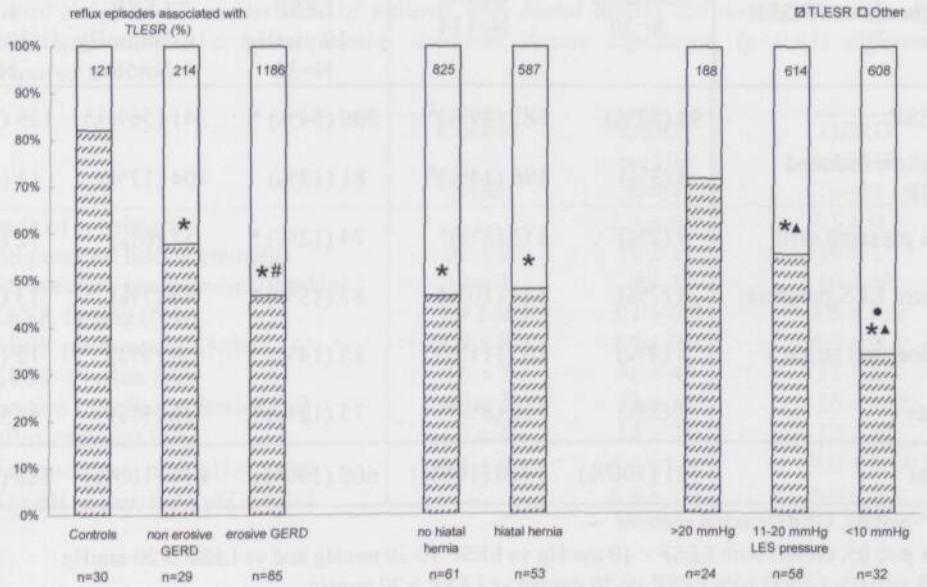
TLESR	Control n=30	GERD n=114	Grade 0 n=29	Grade I N=30	Grade II n=40	grade III n=15
Fasting	2.8±0.4	3.3±0.3	3.8±0.6	2.8±0.6	3.8±0.6	2.2±0.7
Postprandial I	6.1±0.6•	4.6±0.3•*	5.0±0.6	4.3±0.6•	5.0±0.5	3.3±0.6*
II	5.0±0.4•	4.1±0.3	4.2±0.6	3.5±0.5	4.7±0.5	3.5±1.1
III	3.7±0.5	3.2±0.3	3.0±0.5	3.0±0.5	3.5±0.5	3.0±1.0
TLESR associated with reflux (%)	16±3	42±3*	29±3Δ	41±6Δ	47±5Δ	58±7Δ

Mechanism of gastroesophageal reflux

Gastroesophageal reflux during the test occurred in 107 of 114 patients with GERD (94%) and in 22 of 30 healthy controls (79%). Patients with GERD had significantly more reflux compared to controls during the test: 9.0 ± 1.0 vs 0.4 ± 0.1% (p<0.0001).

The major mechanism of reflux both in healthy subjects and reflux patients was TLESR, in 82% and 49% respectively (Table 2). The presence of a hiatal hernia did not significantly affect the mechanisms of reflux. (Figure 3). In patients with erosive reflux disease the contribution of TLESRs to reflux was slightly lower compared to non-erosive reflux disease (46% vs 58%; p<0.05). Reflux mechanisms were significantly related to basal LES pressure. Patients with low LES pressure (≤10 mmHg) had a significantly lower percentage of reflux episodes caused by TLESRs compared to reflux patients with intermediate or normal LES pressure. Table 2 shows in detail the mechanisms by which reflux occurred in GERD patients according to basal level of LES pressure.

Figure 3. *Mechanisms of gastroesophageal reflux in healthy controls and patients with GERD. Patients are divided in subgroups according to presence of erosive GERD, presence of hiatal hernia and fasting LES pressure. Asterisks denote significant differences vs. controls (p<0.001). The fence denotes a significant difference between erosive vs. non-erosive GERD (p<0.01). The triangles denote significant differences vs. GERD subgroup with normal LESP (p<0.001). The dot denotes a significant difference between GERD subgroup with low LESP vs. intermediate LESP (p<0.001).*



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TLESR	Control	GERD	Grade 0	Grade I	Grade II	grade III
	n=30	n=114	n=29	N=30	n=40	n=15
Fasting	2.8±0.4	3.3±0.3	3.8±0.6	2.8±0.6	3.8±0.6	2.2±0.7
Postprandial I	6.1±0.6•	4.6±0.3•*	5.0±0.6	4.3±0.6•	5.0±0.5	3.3±0.6*
II	5.0±0.4•	4.1±0.3	4.2±0.6	3.5±0.5	4.7±0.5	3.5±1.1
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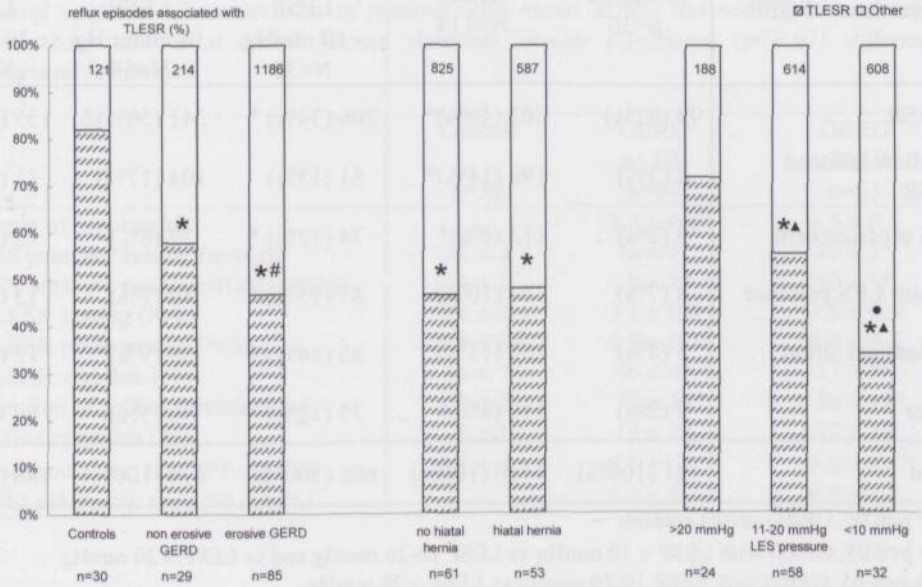

Table 2. *Mechanisms of gastroesophageal reflux for all reflux episodes observed during a total recording time of 474 hours in 30 healthy volunteers and 114 patients with gastroesophageal reflux disease. GERD patients were divided in 3 groups based on basal LES pressure: < 10 mmHg (low pressure), 10-20 mmHg (intermediate pressure) and > 20 mmHg (normal pressure).*

Mechanisms of GER	Controls n=30	GERD N=114	GERD		
			LESP <10 mmHg N=32	LESP 10-20mmHg N=58	LESP >20 mmHg N=24
TLESR	99 (82%)	682 (49%)*	206 (34%) •	341 (56%)Δ	135 (72%)
Swallow induced LESR	4 (3%)	196 (14%)*	81 (13%)	104 (17%)	11 (6%)
LES pressure drift	3 (2%)	113 (8%)*	74 (12%) •	37 (6%)	12 (6%)
Absent LES pressure	8 (7%)	144 (10%)	87 (15%) •	44 (7%)	13 (7%)
Abdominal strain	5 (4%)	155 (11%)*	85 (14%) •	58 (9%)	12 (6%)
Other	2 (2%)	110 (8%)*	75 (12%) •	30 (5%)	5 (3%)
Total	121 (100%)	1410 (100%)	608 (100%)	614 (100%)	188 (100%)

* $p<0.05$, GERD versus controls

• $p<0.05$, GERD with LESP < 10 mmHg vs LESP 10-20 mmHg and vs LESP > 20 mmHg

Δ $p<0.05$, GERD with LESP 10-20 mmHg vs LESP > 20 mmHg

Hiatal Hernia

Reflux mechanisms were not significantly different between reflux patients without and with a hiatal hernia (Table 3). In addition, neither were grade of esophagitis, LES pressure or TLESR frequency different between refluxers with and without a hiatal hernia.

Table 3. *Characteristics of GERD patients with or without hiatal hernia (HH) compared to control subjects. Characteristics of patients with hiatal hernia did not significantly differ from those without a hiatal hernia. Asterisks denote significant (p<0.01) differences compared to control.*

	Control n=30	GERD no HH n=61	GERD with HH n=53
Grade of esophagitis	-	1.3 ± 0.1	1.5 ± 0.1
LES pressure fasting (mmHg)	22 ± 2	16 ± 1*	16 ± 1*
LES pressure postprandial (mmHg)	14 ± 1	9 ± 1*	10 ± 1*
TLESR fasting (N/h)	2.8 ± 0.4	3.1 ± 0.4	3.5 ± 0.5
TLESR postprandial (N/h)	4.9 ± 0.4	4.5 ± 0.3	4.4 ± 0.4
TLESR + reflux (%)	16 ± 3	46 ± 4*	37 ± 5*
Duration of reflux episode (sec)	10 ± 2	73 ± 14*	56 ± 11*
Reflux episodes (N)	3 ± 1	15 ± 2*	12 ± 2*
Test procedure: time pH < 4 (%)	0.4 ± 0.1	9.1 ±1.8*	9.0 ± 2.1*
24 hr pH metry: time pH < 4(%)	—	9.8 ± 1.3	9.0 ± 1.2

DISCUSSION

We investigated the contribution of TLESRs to reflux in a large cohort of patients with GERD and healthy controls. In healthy controls, reflux to a small extent, is physiological and does not lead to symptoms. In the control subjects over 80% of the reflux episodes were provoked by TLESRs. Under postprandial conditions the frequency of TLESRs in healthy controls increases, however this increase in number of TLESRs does not greatly affect reflux time because only a small percentage of TLESRs was accompanied by reflux (16 ± 3%).

Patients with reflux disease did not have more TLESRs compared to controls as has been suggested previously (2-4, 6). TLESR frequency in reflux patients was not different from controls, as has been shown in several studies (7, 15-21). More precisely, in the first postprandial hour TLESR frequency even was significantly lower than in controls. TLESRs are a physiological phenomenon, especially in the postprandial period. During TLESRs venting of (excess) gas and/or liquid from the stomach is allowed. In the GERD patients the percentage of TLESRs associated with acid reflux was significantly higher compared to controls. Our data are in line with several studies on reflux and TLESRs, all reporting a

higher incidence of acid reflux during TLESRs in patients with GERD. Reflux of gas can be differentiated from liquid/acid reflux by electrical impedance measurement. As has been shown by Sifrim et al., many TLESRs are associated with liquid, non-acidic, reflux (19, 22).

Our data obtained in a large cohort of GERD patients settle account with the concept that reflux disease results from a disordered control of triggering of TLESRs. With increasing severity of endoscopic esophagitis more TLESR become associated with reflux. In patients with severe esophagitis even 58% of TLESR were accompanied by reflux.

The presence of a hiatal hernia did not influence reflux severity or reflux mechanism within the group of GERD patients. In patients with or without a hiatal hernia, about 50% of reflux episodes were associated with TLESRs and about 20% with low or absent LES pressure. Kahrilas et al. (23) studied GERD patients with and without a hiatal hernia by gastric distension with air and found a positive correlation between frequency of TLESRs and presence of a hiatal hernia. The separation of the lower esophageal sphincter from the diaphragm in case of hiatal hernia may facilitate TLESR triggering in the supine position. In upright position, hiatal hernia had no impact on TLESR frequency (9), an observation that is in line with our results. In an earlier report from the group of Kahrilas (24) a model on acid reflux was designed comprising data as LES pressure, size of hiatal hernia and reflux score. Both LES pressure and the length of a hiatus hernia were determinants of gastroesophageal junction incompetence. Van Herwaarden et al. (9) studied patients with or without a hiatal hernia by 24 hr ambulatory manometry of the LES and by pH metry. Patients with a hiatus hernia had more reflux but the excess of reflux in the patients with a hiatal hernia was not due to higher frequency of TLESR but due to other mechanisms of reflux such as low LES pressure, pointing to malfunction of the gastroesophageal barrier. The outcome of that study may have been affected by patient selection because only small numbers of reflux patients were investigated (9). In our large cohort of GERD patients the presence of a hiatal hernia did not influence the amount of acid reflux, nor reflux mechanisms nor LES motor characteristics. Hiatal hernia, however, prolonged the duration of reflux episodes. This may result not only from a higher acid volume load to the esophagus but also from impaired esophageal acid clearance.

The frequency of TLESRs was shown to be dependent on basal LES pressure. Patients with GERD with a normal LES pressure had significantly more TLESRs vs patients with low LES pressure. In the GERD patients with normal LES pressure 72% of reflux episodes were caused by TLESRs. In patients with low LES pressure TLESR frequency was reduced compared to controls and in that subset only 34% of reflux episodes occurred during TLESRs. The level of fasting LES pressure is therefore an important predictor of the reflux pattern in an individual GERD patient.

Our data suggest that different types of refluxers exist: patients with TLESR predominant reflux on the one hand and patients with esophagogastric junction incompetence on the other hand. In the later group, LES drift or absent pressure or abdominal strain (together causing 41% of reflux episodes) dominate over TLESRs (34% of reflux episodes).

Medical therapy of reflux disease is directed towards reducing the acidic component of the refluxate. This therapy is usually very effective but may fail in a small subset of patients (non responders) (25, 26). It is clinically relevant to determine the type of refluxer in the group of non responders because patients with low LES pressure and sphincter malfunction may require a treatment strategy that is different from patients with normal LES

pressure and TLESR predominant reflux. Future research should focus therefore more on quality than on quantity of TLESRs.

It is concluded that: 1) in GERD patients, frequency of TLESRs in the fasting state is not different form controls and postprandially the TLESR frequency even is significantly reduced. 2) in GERD patients significantly more TLESRs are associated with acid reflux. 3) in GERD patients TLESRs are the major mechanism of reflux but subtypes of refluxers exist (TLESR predominant vs LES incompetence). 4) LES pressure and not hiatal hernia or endoscopic grade of esophagitis determine the reflux mechanism.

REFERENCES

1. Dent J, Dodds WJ, Friedman RH, et al. Mechanism of gastroesophageal reflux in recumbent asymptomatic human subjects. J Clin Invest 1980;65:256-267.
2. Dodds WJ, Dent J, Hogan WJ, et al. Mechanisms of gastroesophageal reflux in patients with reflux esophagitis. N Engl J Med 1982;307:1547-1552.
3. Mittal RK, Holloway RH, Penagini R, et al. Transient lower esophageal sphincter relaxation. Gastroenterology 1995;109:601-610.
4. Holloway RH, Kocyan P, Dent J. Provocation of transient lower esophageal sphincter relaxations by meals in patients with symptomatic gastroesophageal reflux disease. Dig Dis Sci 1991;36:1034-1049.
5. Holloway RH, Lyrenas E, Ireland A. et al. Effect of intraduodenal fat on lower oesophageal sphincter function and gastro-oesophageal reflux. Gut 1997;40:449-453.
6. Penagini R, Bianchi PA. Effect of morphine on gastroesophageal reflux and transient lower esophageal sphincter relaxation. Gastroenterology 1997;113:409-414.
7. Schoeman MN, Tippett MD, Akkermans LM, et al. Mechanisms of gastroesophageal reflux in ambulant healthy human subjects. Gastroenterology 1995;108:83-91.
8. Dent J, Holloway RH, Toouli J, et al. Mechanisms of lower oesophageal sphincter incompetence in patients with symptomatic gastrooesophageal reflux. Gut 1988;29:1020-1028.
9. Van Herwaarden MA, Samson M, Smout AJ. Excess gastroesophageal reflux in patients with hiatus hernia is caused by mechanisms other than transient LES relaxations. Gastroenterology 2000;1119:1439-1446.
10. Savary M and Miller G. The esophagus. Handbook and Atlas of endoscopy. Solothurn, Switzerland: Gassmann AG, 1978:135-139.
11. Masclee AAM, de Best ACAM, de Graaf R, et al. Ambulatory 24 hr pH metry in the diagnosis of gastroesophageal reflux disease. Scand J Gastroenterol 1990;25:225-230.
12. Holloway RH, Penagini R, Ireland AC. Criteria for objective definition of transient lower esophageal sphincter relaxation. Am J Physiol 1995;268:G128-G133.
13. Smout AJ, Breedijk M, van der Zouw C, et al. Physiological gastroesophageal reflux and esophageal motor activity studied with a new system for 24-hour recording and automated analysis. Dig Dis Sci 1989;34:372-378.
14. Richter JE. Gastroesophageal reflux disease in the older patient: presentation, treatment, and complications. Am J Gastroenterol 2000;95:368-373.
15. Kahrilas PJ, Gupta RR. Mechanisms of acid reflux associated with cigarette smoking. Gut 1990;31:4-10.
16. Penagini R, Mangano M, Bianchi PA. Effect of increasing the fat content but not the energy load of a meal on gastro-oesophageal reflux and lower oesophageal sphincter motor function. Gut 1998;42:330-333.
17. Lidums I, Lehmann A, Checklin H, et al. Control of transient lower esophageal sphincter relaxations and reflux by the GABA(B) agonist baclofen in normal subjects. Gastroenterology 2000;118:7-13.
18. Holloway RH, Zhang Q. The GABA-B receptor agonist Baclofen inhibits transient lower oesophageal sphincter relaxations and gastro-oesophageal reflux in patients with reflux disease. Gut 2000;47:A24.
19. Sifrim D, Holloway R, Silny J, et al. Composition of the postprandial refluxate in patients with gastroesophageal reflux disease. Am J Gastroenterol 2001;96:647-755.
20. Trudgill NJ, Riley SA. Transient lower esophageal sphincter relaxations are no more frequent in patients with gastroesophageal reflux disease than in asymptomatic volunteers. Am J Gastroenterol 2001;96:2569-2574.

21. Wong WM, Lai KC, Hui WM, et al. Pahtophysiology of gastroesophageal reflux diseases in Chinese – Role of transient lower esophageal sphincter relaxation and esophageal motor dysfunction. Am J Gastroenterol 2004;99:2088-2093.
22. Sifrim D, Silny J, Holloway RH, et al. Patterns of gas and liquid reflux during transient lower esophageal sphincter relaxation. A study using intraluminal electrical impedance. Gut 1999;44:47-54.
23. Kahrilas PJ, Shi G, Manka M, et al. Increased frequency of transient lower esophageal sphincter relaxation induced by gastric distension in reflux patients with hiatal hernia. Gastroenterology 2000;118:688-695.
24. Sloan S, Rademaker AW, Kahrilas PJ. Determinants of gastroesophageal junction incompetence: hiatal hernia, lower esophageal sphincter, or both? Ann Int Med 1992;117:977-982.
25. Kahrilas PJ. Gastroesophageal reflux disease. JAMA 1996;276:983-988.
26. Chiba N, DeGara CJ, Wilkinson JM, et al. Speed of healing and symptom relief in grade II to IV gastroesophageal reflux disease: a meta-analysis. Gastroenterology 1997;112:1798-1810.

3

PROVOCATION OF TRANSIENT LOWER ESOPHAGEAL SPHINCTER RELAXATIONS BY GASTRIC DISTENSION WITH AIR

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Am J Gastroenterol 2001;96:2317-2323.

ABSTRACT

Background: Transient lower esophageal sphincter relaxations (TLESRs) are the major mechanism permitting not only gastroesophageal reflux but also venting of air from the stomach. Triggering of TLESRs is provoked by gastric distension. Antireflux surgery is associated with impaired ability to belch. It is not known whether a reduced capacity to belch results from postoperative reduction in TLESRs.

Methods: We studied the occurrence of TLESRs, common cavities (indicator for gas gastroesophageal reflux) and belching after standardized acute gastric distension by air insufflation (750 ml). Control subjects (n = 10), patient with GERD (n = 22) and patients after fundoplication (n = 24) were studied. LES and esophageal motility were recorded with perfusion manometry (Dent-sleeve).

Results: Gastric distension with air significantly ($p<0.05$) increased TLESR frequency in controls (1.6 ± 0.3 to 3.5 ± 1.0 per 20 min), GERD patients (1.2 ± 0.3 to 3.1 ± 0.5 per 20 min) and post fundoplication (0.5 ± 0.1 to 1.8 ± 0.6 per 20 min). Post fundoplication the number of TLESRs was significantly ($p<0.05$) reduced both under fasting conditions and after air insufflation. The number of common cavities and belches after gastric air distension also was significantly ($p<0.05$) reduced post fundoplication: 2.3 ± 0.6 versus 4.7 ± 0.4 in controls and 4.1 ± 0.4 in GERD patients. About half of the common cavities occurred during TLESRs, half during other mechanisms. An impaired ability to belch in daily life correlated with an impaired belching response during the test. An impaired ability to belch occurred only in patients with complete and not in patients with partial fundoplication and was associated with a reduced number of common cavities after gastric air insufflation.

Conclusions: short lasting gastric air distension 1) provokes TLESRs but does not differentiate GERD patients from controls; 2) reveals impaired belching capacity in patients after complete fundoplication; 3) shows that common cavities do not exclusively occur during TLESRs.

INTRODUCTION

It has been well established that transient lower esophageal sphincter relaxations (TLESRs) are the major mechanism permitting gastroesophageal reflux in man (1). Patients with gastroesophageal reflux disease (GERD) are believed to have a higher frequency of TLESRs compared to controls (2,3). Other investigators have pointed to an increased percentage of TLESRs associated with acid reflux in GERD patients (1,4). Reflux occurs especially in the postprandial periods (5). After meal ingestion the number of TLESRs increases depending on the size of the meal and meal composition. Gastric distension, especially of the fundus, is a trigger for TLESRs (6,7). To study TLESRs in patients with GERD, prolonged intraluminal recording of pressure and pH after meal ingestion is necessary. The aim of this study is to explore whether a short-duration provocation test with gastric distension by air insufflation is able to detect differences in TLESRs between GERD patients and controls.

TLESRs are also considered the physiological mechanism allowing venting of excess gas from the stomach (8,9). TLESRs underlie virtually all episodes of gas reflux during belching. After fundoplication the frequency of TLESRs decreases significantly (10).

Antireflux surgery is associated with impaired ability to belch, abdominal bloating, and increased flatulence. It is not known whether a reduced capacity to belch results from postoperative reduction in TLESRs. Gastric distension with air allows us to investigate both the presence of TLESRs, frequency of belching and their correlation in patients after fundoplication.

METHODS

Subjects

Three groups were studied including in total 56 subjects:

1) Healthy volunteers (n=10; 6 females, 4 males; age 20 - 48 years). None of them had a history of gastro-intestinal disease, reflux symptoms or previously underwent surgery or was on chronic medication.

2) Patients with GERD (n=22; 8 females, 14 males; age 27 - 59 years). Gastroesophageal acid reflux in these patients was proven by endoscopy (esophagitis) or by 24 hour ambulatory pH metry. A hiatal hernia was present in 12 subjects with mean length 4.3 ± 0.4 cm. All patients underwent 24 hour pH metry and total time pH<4 ranged from 0.9 to 48.5 % (mean ± SEM: 10.5±2.6%; normal value <4.0%).

3) Patients after laparoscopic antireflux surgery (n=24; 11 females, 13 males; age 31 - 76 years). Fourteen patients underwent a complete (360o) Nissen fundoplication. Ten patients underwent a partial (180°-270°) fundoplication. Postoperatively the percentage of time esophageal pH<4 during 24 hour pH metry ranged from 0 to 11.7% (mean ± SEM: 3.7±0.8%).

Informed consent was obtained from each individual. The study had been approved by the Ethics Committee of the Leiden University Medical Center.

Manometric and pH technique

The manometry catheter consisted of a multilumen silicone tube (outer diameter 5.0 mm) with seven side holes located at 29, 23, 18, 13, 8, 3 and -4 cm from the mid of the 6 cm long sleeve sensor (Dentsleeve Pty Ltd, Belair, South Australia). The catheter was continuously perfused with gas free distilled water by a low compliance pneumohydraulic capillary infusion system (Arndorfer Medical Specialties, Greendale, Wisconsin, U.S.A.) at a rate of 0.5 ml/min. The external pressures transducers (Medex Inc., Ohio, U.S.A.) were connected via an analogue/digital converter (PC Polygraph HR, Synectics Medical, Stockholm, Sweden) to a personal computer system. The data were displayed continuously on a monitor and stored on the personal computer system (Polygram Upper GI 6.30, Gastrosoft Inc., Synectics Medical, Stockholm, Sweden).

The manometry catheter was introduced through the nose into the esophagus and positioned so that the sleeve sensor straddled the LES. The proximal side hole was positioned in the pharynx and was used for identification of swallow signals. The middle side holes registered esophageal body motility. The distal side hole was used as reference point for intragastric pressure. A glass pH electrode (Ingold LOT 440 continue glassreference electrode; Ingold Messtechnik AG, Urdorf, Germany) was passed through the nose and positioned 5 cm above the upper margin of the LES. The pH electrode had been calibrated at pH 4.0 and pH 7.0.

Study protocol

The experiments were started at 9.00 a.m. after an overnight fast. The subjects were studied in the upright position, sitting in a comfortable chair. The manometry and pH catheter were introduced into the esophagus and positioned as described above. Esophageal pH and motility were registered simultaneously for one hour under basal, fasting conditions (time 0 to 60 min) and during two 20 min periods after air insufflation. Air was insufflated into the stomach at time 60 min and at time 80 min in 750 ml portions insufflated in 2 min through the distal intragastric side hole of the manometry assembly using a 60 ml syringe. The occurrence of TLESRs, common cavities and belches (definition see below) was scored in 20 min periods (basal, air insufflation). If the subject perceived gastropharyngeal reflux of air (belching) a mark was set on the computer tracing. If necessary, remaining air was removed from the stomach, at the end of the study protocol.

Lower esophageal sphincter data analysis

Lower esophageal sphincter tracings were analyzed for LES resting pressure (LESP) and LES relaxations (LESR). LESP was defined as mean end-expiratory LESP relative above intragastric pressure. LESR are divided in swallow induced LESR and spontaneous LESR. Swallow induced LESR are preceded by active swallows starting with a pharyngeal contraction. Spontaneous LESR, better known as transient LES relaxation (TLESR) are divided in non-swallow related TLESR, and swallow related TLESR. Spontaneous, non swallow related TLESR are defined as decreases in LESP of ≥ 5 mmHg with a rate of ≥ 1 mmHg/sec, within 10 sec reaching a pressure of ≤ 2 mmHg above intragastric pressure. No swallow signal occurs in the interval from 4 sec before to 2 sec after onset of LESR. Swallow related TLESR are defined as spontaneous TLESR, irrespective of the timing of LESR to swallowing when the duration of LESR is at least 10 sec (1).

Gastroesophageal gas or acid reflux

Common cavities were defined as abrupt simultaneous and sustained rises of basal esophageal pressure to intragastric pressure in at least the two lower esophageal body manometry recording sites (11). Common cavities are considered as markers of gas (or liquid) reflux from the stomach into the esophagus. Belching consists of gas gastroesophageal reflux (common cavity) followed by gas esophagopharyngeal reflux. We considered the occurrence of common cavities as indication for gas gastroesophageal reflux during the distension experiments. The belching response was considered impaired when ≤1 common cavity per 20 min air insufflation period was observed. The mechanisms of each gas or acid reflux episode was scored using previous described criteria (12).

Symptom analysis

Subjects were asked for symptoms of bloating and the ability to belch. These symptoms were scored on a scale from 0 to 3 (0 = no symptoms, able to belch; 1 = mild symptoms; 2 = moderate symptoms; 3 = severe symptoms, not able to belch).

Statistical analysis

Data are expressed as mean ± SEM. Data were analyzed for statistical significance using (multiple) analysis of variance to compare results between the groups of patients and the controls. When this indicated a probability of less than 0.05 for the null hypothesis,

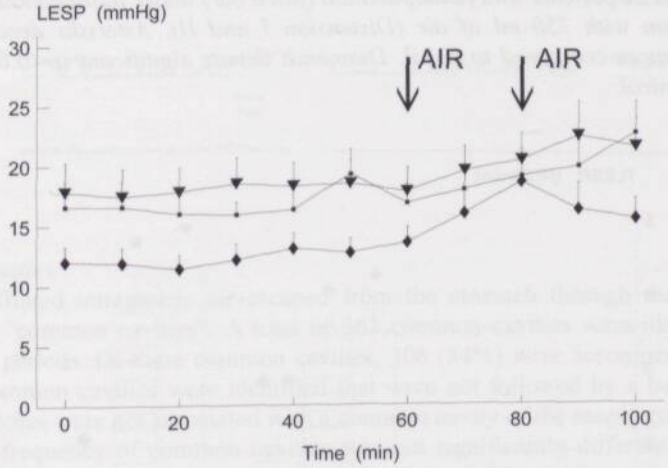
Student-Newman-Keuls analyses were performed to determine which values between or within the experiments differed significantly. A p value of <0.05 was considered significant for all analyses.

RESULTS

Lower esophageal sphincter pressure

LES pressure was significantly ($p<0.05$) higher in patients after fundoplication (18 ± 2 mmHg) compared to patients with GERD the (11 ± 1 mmHg), but not compared to controls (16 ± 2 mmHg). In patients with GERD the LES pressure was significantly lower ($p<0.05$) compared to controls. Intragastric insufflation of air resulted in small, non-significant increases in LES pressure (Figure 1).

Figure 1. *Lower esophageal sphincter pressure (mean ± SEM) in 10 healthy controls (small squares), 22 patients with gastroesophageal reflux disease (diamonds) and 24 patients after fundoplication (triangles) under basal fasting conditions and during gastric distension with air.*



Transient lower esophageal sphincter relaxations

The frequency of TLESRs in the basal state was not significantly different between patients with GERD and controls (1.2 ± 0.3 vs 1.6 ± 0.3 per 20 min). Patients after fundoplication had a significantly ($p<0.05$) lower frequency of TLESRs (0.6 ± 0.1 per 20 min) compared to controls (Figure 2). Gastric distension with 750 ml air significantly ($p<0.05$) increased the frequency of TLESRs in the control group (to 3.5 ± 1.0 per 20 min), in patients with GERD (to 3.1 ± 0.5 per 20 min) and in patients after fundoplication (to 1.8 ± 0.6 per 20 min). The number of TLESRs was not significantly different between the first and second distension procedure. Although the frequency of TLESRs in response to distension increased in patients with fundoplication it remained significantly ($p<0.05$) reduced compared to control subjects and patients with GERD. The frequency of TLESRs was not significantly different between patients with a complete fundoplication compared to patients with a partial fundoplication, neither basally (0.6±0.2 versus 0.5±0.2 per 20 min) nor after gastric distension (1.9±0.8 versus 1.4±0.5 per 20 min). The presence of a hiatal hernia in patients with GERD did not significantly influence the frequency of TLESR: 3.1±0.8 versus 3.1±0.7 per 20 min (hiatal hernia versus no hiatal hernia).

Figure 2. *Frequency of transient lower esophageal sphincter relaxations (N/20 min; mean ± SEM) in 10 healthy controls (open bar), 22 patients with gastroesophageal reflux disease (shaded bar) and 24 patients with fundoplication (filled bar) under basal conditions and after gastric distension with 750 ml of air (Distension I and II). Asterisks denote significant ($p<0.05$) differences compared to basal. Diamonds denote significant ($p<0.05$) differences compared to control.*

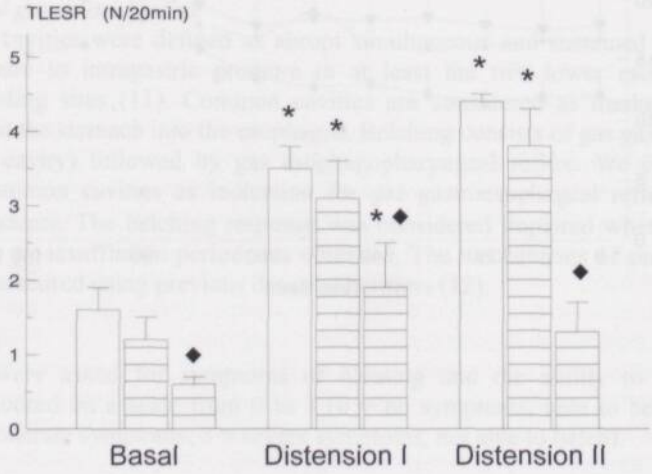
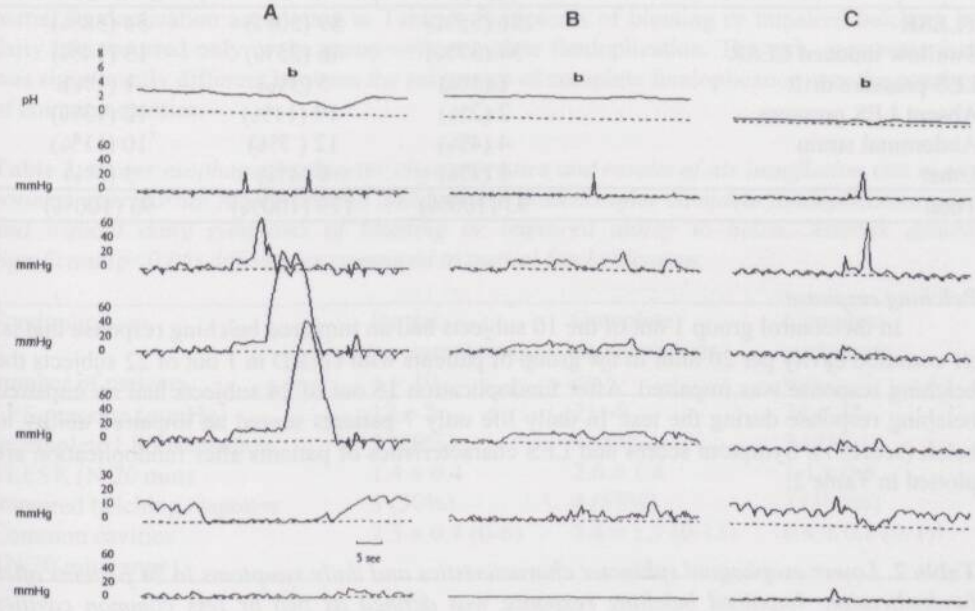


Figure 3. *Examples of common cavities during a transient LES relaxation (A); during low LES pressure (B) and after abdominal straining (C) (b = belch. Upper line: esophageal pH; 2nd line: pharynx; 3rd - 5th line: esophageal motility (5 cm intervals); 6th line: LES (sleeve); distal line: stomach.*



Common cavities

Insufflated intragastric air escaped from the stomach through the esophagogastric junction by "common cavities". A total of 365 common cavities were identified during all insufflation periods. Of these common cavities, 308 (84%) were accompanied with a belch. Thus, 57 common cavities were identified that were not followed by a belch. On the other hand, 37 belches were not associated with a common cavity of the esophageal body.

The frequency of common cavities was not significantly different between patients with GERD and controls (4.1 ± 0.4 versus 4.7 ± 0.4 per 20 min distension period). Patients after fundoplication had significantly ($p<0.05$) less common cavities (2.3 ± 0.6 per 20 min) compared to controls or patients with GERD. The frequency of common cavities was not significantly different between patients with complete or partial fundoplication (2.0 ± 1.0 versus 2.7 ± 0.7 per 20 min).

Common cavities occurred mainly during relaxations of the LES. The main mechanism "permitting" common cavities was a TLESR. However, common cavities also occurred during swallow induced LES relaxation, episodes of absent LES pressure or abdominal straining (Table 1). TLESRs accounted for 50 to 60% of the common cavities in all groups studied.

Table 1. *Mechanisms of common cavities (total number/40 min) in healthy controls, patients with gastroesophageal reflux disease and patients with fundoplication during two successive gastric distensions with 750 ml air.*

	Control n=10	GERD n=22	Fundoplication n=24
TLESR	51 (55%)	89 (50%)	54 (58%)
Swallow induced LESR	34 (37%)	46 (25%)	13 (14%)
LES pressure drift	1 (1%)	5 (3%)	1 (1%)
Absent LES pressure	2 (2%)	19 (11%)	12 (13%)
Abdominal strain	4 (4%)	12 (7%)	10 (11%)
Other	1 (1%)	8 (4%)	3 (3%)
Total	93 (100%)	179 (100%)	93 (100%)

Belching response

In the control group 1 out of the 10 subjects had an impaired belching response that is: ≤1 common cavity per 20 min. In the group of patients with GERD in 1 out of 22 subjects the belching response was impaired. After fundoplication 16 out of 24 subjects had an impaired belching response during the test. In daily life only 7 patients scored an impaired ability to belch (score≥1). Symptom scores and LES characteristics of patients after fundoplication are plotted in Table 2.

Table 2. *Lower esophageal sphincter characteristics and daily symptoms in 24 patients after fundoplication. Impaired belching response was defined as two or less common cavities during two successive intragastric insufflations of 750 ml of air in 40 min. Asterisks denote significant (p< 0.05) differences compared to normal.*

Belching response number of patients	Normal n = 8	Impaired n = 16
Complete fundoplication (Nissen)	3 (37%)	11 (69%)
Daily life symptoms:		
bloating (score≥1)	0 (0%)	4 (25%)
impaired belching (score≥1)	0 (0%)	7 (44%)*
LES pressure (mmHg)	11 ± 3	22 ± 2*
Incomplete LES relaxation (N)	1 (13%)	8 (50%)*
TLESR (N/20 min)	3.5 ± 1.2	0.8 ± 0.3*
Common cavities (N/20 min;range)	5.6 ± 1.1 (3-13)	0.4 ± 0.1* (0-1)

Compared to patients after fundoplication with a normal belching response in the test, in patients with an impaired belching response in the test the LES pressure was significantly higher, LES relaxation after wet swallows was frequently incomplete and the number of TLESR was significantly lower. Eleven patients (69%) with an impaired belching response in the test had a complete Nissen fundoplication in contrast to the group of patients with a normal belching response in the test (37%). LES characteristics of patients with a complete or partial fundoplication are plotted in Table 3. Symptoms of bloating or impaired belching in daily life occurred only in the group with complete fundoplication. The only parameter that was significantly different between the subgroups of complete fundoplication was the number of common cavities.

Table 3. *Lower esophageal sphincter characteristics and results of air insufflation test in ten patients after partial fundoplication and fourteen patients after complete fundoplication with and without daily symptoms of bloating or impaired ability to belch. Asterisk denote significant (p<0.05) differences compared to partial fundoplication.*

Fundoplication	Partial	Complete	Complete
	no symptoms	no symptoms	symptoms
number of patients	n = 10	n=7	n = 7
LES pressure (mmHg)	12 ± 2	21±4	24 ± 3*
Incomplete LES relaxation	2 (20%)	4 (57%)	3 (43%)
TLESR (N/20 min)	1.4 ± 0.4	2.6 ± 1.4	1.1 ± 0.5
Impaired belching response	5 (50%)	4 (57%)	7 (100%)
Common cavities (N/20 min;range)	2.5 ± 0.7 (0-6)	3.4 ± 1.7 (0-13)	0.4 ± 0.1 (0-1)*

Mechanisms of gastro-esophageal acid reflux

Under fasting conditions and after gastric distension the number of reflux episodes and the amount of time pH < 4 were very low in the healthy volunteers (1.1 episodes per subject per hour; 0.33% time pH < 4), in patients with GERD (1.3 episodes per subject per hour; 0.97% time pH < 4) and patients after fundoplication (1.0 episodes per hour; 0.88% time pH <4).

DISCUSSION

Acute gastric distension of the proximal stomach with air significantly increases the frequency of TLESRs. Patients with GERD were not different from healthy controls with respect to the frequency of TLESRs. However, in patients after antireflux surgery significantly less TLESRs occurred compared to patients with GERD and controls. A large subgroup of patients after fundoplication had an impaired belching response after gastric distension with air. These patients were characterized by regular symptoms of bloating and impaired belching, had significantly less TLESRs and were confined to the group with total fundoplication. The occurrence of TLESRs after fundoplication is therefore an important mechanism preventing bloating after antireflux surgery.

As expected, basal LES pressure in patients with GERD was lower than in the controls. In the patients after antireflux surgery LES pressure was significantly higher. During gastric distension with air LES pressure increased, although not significantly. This finding is in line with previous studies both in man and in animals reporting small increments in LESP in response to gastric distension (6,7,13,14).

Gastric distension with air resulted in increases in the frequency of TLESRs both in healthy subjects, patients with GERD and patients after antireflux surgery. However, in patients after fundoplication the frequency of TLESRs was significantly reduced compared to GERD and controls. Surprisingly, the number of TLESRs after air provocation was not higher in patients with GERD compared to controls. Several investigators (7,9,13,15,16) have shown that gastric distension with air increases the frequency of TLESRs. However, the effect of air insufflation is only short lasting because with the occurrence of TLESRs air escapes through belching and there after the distension of the stomach rapidly diminishes. It is not clear whether patients with GERD have an altered TLESR frequency after gastric distension. A previous study reported that patients with GERD have a significantly higher basal frequency of TLESRs compared to healthy subjects (7). However, gastric distension in that study was performed using an intragastric balloon and in contrast to air insufflation, the air could not escape from the balloon.

Numerous studies have shown that ingestion of meal increases the frequency of TLESRs (2,4,17-19). However, these studies have not clearly shown that patients with GERD truly have more TLESRs than controls. The percentage of TLESRs associated with acid reflux however, is increased in patients with GERD compared to controls. It remains to be elucidated why some TLESRs are accompanied with reflux and others are not. Intraluminal electrical impedance measurement has been shown to discriminate between liquid and gas gastroesophageal reflux and may prove a useful tool to answer these questions (20).

We have considered common cavities as markers of gas gastroesophageal reflux. The volume of belched air was not quantified, but is was a consistent finding that the insufflated air was released from the stomach during 3 to 4 common cavities. Half of the common cavities occurred during TLESRs. However a substantial number of common cavities occurred during swallow related LES relaxations or other mechanisms. Air can escape from the stomach through TLESRs but also by other mechanism. This is in line with the findings of studies on mechanism of acid reflux that although TLESRs are the predominant mechanism permitting acid reflux, that reflux of acidic gastric content occurs by other mechanism in 40 to 50%.

After antireflux surgery not only the number of TLESRs was diminished, but also the numbers of common cavities and belches were significantly less compared to GERD patients or controls. Smith et al have studied belching ability in patients after antireflux surgery and normal volunteers (21). After fundoplication patients had per belch lower volumes of expelled air compared to healthy subjects, but the belching frequency was similar. In the group of patients we studied different types of operation have been performed. With respect to TLESR frequency or numbers of common cavities patients with partial fundoplication were not significantly different from patients with complete fundoplication. However, a subgroup of patients with complete fundoplication had significantly less common cavities after air insufflation. These patients did have daily symptoms of bloating and an impaired ability to belch. A higher LES resting pressure, was the only parameter that was significantly different between the patients after complete fundoplication with impaired belching response in the test and impaired belching response in daily life versus those without an impaired belching response in daily life.

Based on the results of the air insufflation test we differentiated the groups of patients in those with a normal and those with an impaired belching response. Of the patients with an impaired response in the test 50% has troubles with belching in daily life. In the patients with impaired belching response esophageal manometry revealed LES insufficiency (i.e. incomplete LES relaxations and diminished number of TLESRs). Daily symptoms of impaired belching and bloating occurred only in patients with a complete fundoplication. Patients after (complete) fundoplication who did have symptoms had a lower number of common cavities. TLESR are probably neurally mediated since cooling of the vagus nerve and atropine inhibit the frequency of TLESR (9,16,18,22). Antireflux surgery may interfere with cholinergic nerve fibers at the esophagogastric junction. The diminished frequency of TLESR may result from local denervation. The fundic wrap covers the cardia which is a potent zone for triggering of TLESRs. It is therefore possible that the fundoplication prohibits gastric distension induced triggering of TLESRs.

Insufflation with air induces only a short lasting gastric distension because the insufflated air is usually vented by belching. Compared to this method an intragastric bag monitored by a barostat may be a more constant trigger for TLESRs. Prolonged gastric distension may discriminate between GERD and healthy subjects with respect to the triggering of TLESRs. After antireflux surgery we found that our test results and symptoms correlated, that is, all patients with postoperative bloating and an impaired ability to belch, had during the test procedure an impaired belching response. On the other hand, an impaired belching response during the test occurred frequently in postoperative patients while in daily life no symptoms of bloating or impaired belching are reported. The results of this study are in favor of partial fundoplication with respect to the postoperative symptoms of bloating and impaired ability to belch.

In conclusion: short lasting gastric distension with air is a potent trigger for TLESRs but does not discriminate between patients with GERD and healthy subjects. In patients after fundoplication the frequency of TLESRs and common cavities was significantly reduced. After antireflux surgery intragastric insufflation revealed impaired belching capacity in two-third of the patients while only half of them have symptoms in daily life. Common cavities occur not only during TLESRs but also by other mechanisms.

REFERENCES

1. Mittal RK, Holloway RH, Penagini R, et al. Transient lower esophageal sphincter relaxation. Gastroenterology 1995;109:601-10.
2. Dodds WJ, Dent J, Hogan WJ, et al. Mechanisms of gastroesophageal reflux in patients with reflux esophagitis. N Engl J Med 1982;307:1547-52.
3. Tsimoyiannis EC, Lekkas ET, Berekos K, et al. Modified Nissen fundoplication in the treatment of gastroesophageal reflux disease. Int Surg 1993;78:112-6.
4. Holloway RH, Kocyan P, Dent J. Provocation of transient lower esophageal sphincter relaxations by meals in patients with symptomatic gastroesophageal reflux. Dig Dis Sci 1991;36:1034-9.
5. Dent J, Dodds WJ, Friedman RH, et al. Mechanism of gastroesophageal reflux in recumbent asymptomatic human subjects. J Clin Invest 1980;65:256-67.
6. Franzi SJ, Martin CJ, Cox MR, et al. Response of canine lower esophageal sphincter to gastric distension. Am J Physiol 1990;259:G380-5.
7. Holloway RH, Hongo M, Berger K, et al. Gastric distention: a mechanism for postprandial gastroesophageal reflux. Gastroenterology 1985;89:779-84.
8. McNally EF, Kelly JE, Ingelfinger FJ. Mechanism of belching: effects of gastric distension with air. Gastroenterology 1964;64:254-9.
9. Wyman JB, Dent J, Heddle R, et al. Control of belching by the lower oesophageal sphincter. Gut 1990;31:639-46.
10. Johnsson F, Holloway RH, Ireland AC, et al. Effect of fundoplication on transient lower oesophageal sphincter relaxation and gas reflux. Br J Surg 1997;84:686-9.
11. Holloway RH, Wyman JB, Dent J. Failure of transient lower oesophageal sphincter relaxation in response to gastric distension in patients with achalasia: evidence for neural mediation of transient lower oesophageal sphincter relaxations. Gut 1989 30:762-7.
12. Straathof JWA, Lamers CBHW, Masclee AAM. Effect of gastrin-17 on lower esophageal sphincter characteristics in man. Dig Dis Sci 1997;42:2547-51.
13. Boulant J, Fioramonti J, Dapoigny M, et al. Cholecystokinin and nitric oxide in transient lower esophageal sphincter relaxation to gastric distention in dogs. Gastroenterology 1994;107:1059-66
14. Muller-Lissner SA, Blum AL. Fundic pressure rise lowers lower esophageal sphincter pressure in man. Hepatogastroenterology 1982;29:151-2.
15. Boulant J, Mathieu S, D'Amato M, et al. Cholecystokinin in transient lower oesophageal sphincter relaxation due to gastric distension in humans. Gut 1997;40:575-81.
16. Mittal RK, Chiareli C, Liu J, et al. Atropine inhibits gastric distension and pharyngeal receptor mediated lower oesophageal sphincter relaxation. Gut 1997;41:285-90.
17. Mittal RK, McCallum RW. Characteristics and frequency of transient relaxations of the lower esophageal sphincter in patients with reflux esophagitis. Gastroenterology 1988;95:593-9.
18. Lidums I, Checklin H, Mittal RK, et al. Effect of atropine on gastro-oesophageal reflux and transient lower oesophageal sphincter relaxations in patiens with gastro-oesophageal reflux disease. Gut 1999;43:12-6.
19. Horbach JM, Masclee AAM, Lamers CBHW, et al. A prospective study on the effect of the Belsey Mark IV 270- degree fundoplication on lower esophageal sphincter characteristics and esophageal body motility. J Thorac Cardiovasc Surg 1995;109:636-41.
20. Sifrim D, Silny J, Holloway RH, et al. Patterns of gas and liquid reflux during transient lower oesophageal sphincter relaxation: a study using intraluminal electrical impedance. Gut 1999;44:47-54.
21. Smith D, King NA, Waldron B, et al. F.C. Study of belching ability in antireflux surgery patiens and normal volunteers. Br J Surg 1991;78:32-5.

22. Martin CJ, Patrikios J, Dent J. Abolition of gas reflux and transient lower esophageal sphincter relaxation by vagal blockade in the dog. Gastroenterology 1986;91:890-6.

4

PROVOCATION OF TRANSIENT LOWER ESOPHAGEAL SPHINCTER RELAXATIONS DURING CONTINUOUS GASTRIC DISTENSION

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Scand J Gastroenterol 2002;37:1140-1143.

ABSTRACT

Background: Transient lower esophageal sphincter relaxations (TLESRs) are triggered by gastric distension. Aim of the study was to investigate TLESRs during controlled prolonged gastric distensions using the barostat technique.

Methods: Twelve healthy volunteers (4M; 8F, age range 19-42 yr.) were studied under fasting conditions with combined esophageal manometry (Dentsleeve) and gastric barostat. Randomized isobaric distensions at 0 (control), 10, 12, 14mmHg, were performed each period for 30min with 30min recovery periods in between.

Results: The frequency of TLESR was significantly ($p<0.05$) higher during all distension periods compared to control periods (4.0±0.4 TLESR/30 min vs. 2.6±0.4 TLESR/30min). The frequency of TLESR in the first 15min period of distension was significantly ($p<0.001$) higher compared to the second 15min period pointing to adaptation (2.7±0.3 TLESR/15min vs. 1.3±0.2 TLESR/15min respectively). The frequency of TLESR correlated significantly with intragastric pressure ($r=0.47$; $p<0.01$) and wall tension ($r=0.48$; $p<0.01$), but not with intragastric volume. TLESR characteristics such as duration were not related to pressure or wall tension.

Conclusions: Acute gastric distension increases the frequency of TLESR but adaptation occurs rapidly. The frequency of TLESR during distension is related to pressure and wall tension rather than to intragastric volume.

INTRODUCTION

Transient lower esophageal sphincter relaxations (TLESRs) are triggered by gastric distension (1,2). Under fasting conditions the frequency of TLESR is low, about two relaxations per hour. Ingestion of a meal increases the frequency of TLESR to about 3-6 TLESR per hour in the first postprandial hour, thereafter diminishing in 2-3 hours to basal TLESR frequency depending on the rate of gastric emptying. The volume of meals used in studies for triggering TLESR varied from 150 to 800ml (3-7). Intragastric insufflation of air or carbon dioxide has been used to distend the proximal stomach. Volumes varied from 250 to 1000ml, compatible with small to very large volume meals (8-11). However, the effect of these stimuli diminished over time due to gastric emptying of the ingested meal or belching of the inflated air. Within 10 min after air insufflation most of the air has escaped from the stomach, resulting in gastric distension for only very short period. Intragastric balloon distension enables distensions of the proximal stomach standardized for volume and time (1).

In this study we have used an electronic barostat to stimulate TLESRs. The barostat maintains a constant pressure level by regulation of the volume of inflated air within the intragastric bag (12-14). Using a barostat technique continuous prolonged stimulation of the proximal stomach is performed with fixed intragastric pressures. Aim of the study was to explore the relation between gastric distension and TLESRs.

MATERIALS AND METHODS

Subjects

Twelve healthy volunteers (8 female; age range 19-42 years) participated in a single blind randomized placebo controlled study. Subjects were free of gastrointestinal symptoms and had no history of abdominal surgery. Informed consent was obtained from each individual and the protocol had been approved by the ethical committee of the Leiden University Medical Center.

Lower esophageal sphincter manometry

The manometry catheter consisted of a multilumen silicone rubber tube (outer diameter 5.0mm) with 7 side holes located at 29, 23, 18, 13, 8, 3 and -4cm from the mid-point of the 6cm long sleeve sensor (Dentsleeve Pty Ltd, Belair, South Australia). The catheter was continuously perfused with gas-free distilled water by a low compliance pneumohydraulic capillary infusion system (Arndorfer Med Specialties, Greendale, Wisconsin, U.S.A.) at a rate of 0.5ml/min.

Gastric Barostat

An electronic barostat (Visceral stimulator; Synectics Medical, Stockholm, Sweden) was used to distend the proximal stomach. A polyethylene bag (maximal capacity 1100ml) was tied to the end of a multilumen tube (outer diameter 4.7mm). This catheter was connected to the barostat. The barostat is able to maintain a constant pressure level by an electronic feedback regulation mechanism of the air volume within the intragastric bag. When the stomach contracts, the barostat aspirates air. When the stomach relaxes, the system injects air. The pressure (mmHg) and volume (ml) output of the manometric and barostat recordings were processed by a sixteen channel analogue/digital converter (PC Polygraph HR, Synectics Medical), continuously displayed on one monitor and stored on a personal computer system for later analysis (Polygram for Windows 1.11, Gastrosoft Inc., Synectics Medical).

Study design

The studies were started at 8:30 AM after an overnight fast. The manometry catheter was introduced through the nose into the esophagus and positioned so that the sleeve sensor straddled the LES. The proximal side hole was positioned in the pharynx and was used to record swallow signals. The distal side hole was used as reference point for intragastric pressure. Than the barostat catheter with bag was introduced through the mouth into the fundus. The correct position was checked by fluoroscopy. Subjects were seated in a comfortable chair in a semi-recumbent position with the lower extremities just below abdominal level and supported to prevent abdominal wall straining. LES motility was recorded during the entire study including distension and recovery periods. After a basal period with deflated bag (pressure: 0mmHg), three intermittent isobaric distension periods were performed. The intragastric bag was inflated in randomized order at pressure levels of 10, 12 and 14mmHg for 30min duration. These pressures were chosen because they corresponded with gastric volumes and satiety scores in the postprandial range(18). In between each distension period subjects had a recovery period of 30min. During these periods the bag was deflated (pressure: 0mmHg).

Data analysis

Lower esophageal sphincter tracings were analyzed for resting LES pressure and TLESR with previously described criteria (7,15). Gastric bag volume during distensions is calculated as average values over 5min periods. The first 15min period was compared to the second 15min period of the 30min distensions. Gastric wall tension was calculated by applying Laplace's law: T=P*R/2. Expressing barostat pressure P in mmHg and the length of the radius (R) in centimeters, with the assumption that the intragastric bag is spherical (16).

Statistical analysis

Results are expressed as mean ± SEM. LES pressure data were analyzed for statistical significance using MANOVA. When this indicated a probability of less than 0.05 for the null hypothesis, Student-Newman-Keuls analyses were performed to determine which values between or within the experiments differed significantly. Frequencies of TLESR were analyzed for statistical significance using Mann-Whitney-U test for nonparametric data. A p value of <0.05 was considered significant for all analyses.

RESULTS

Intermittent isobaric distensions

Combined esophageal manometry and gastric barostat distensions were well tolerated by each subject. In each individual a higher intragastric pressure resulted in an increases of bag volume. The intragastric bag pressure was significantly correlated with bag volume (r=0.86; p<0.01). Bag volumes during 30-min distensions at 10mmHg, 12mmHg and 14mmHg were 376±71ml, 464±66ml and 487±65ml respectively. The bag volume of the first 15min period was not significantly different compared to the second 15min period. Wall tension during distension periods of 10mmHg, 12mmHg and 14mmHg was 43±4mmHg*cm, 56±3mmHg*cm and 67±3mmHg*cm respectively.

Lower esophageal sphincter pressure

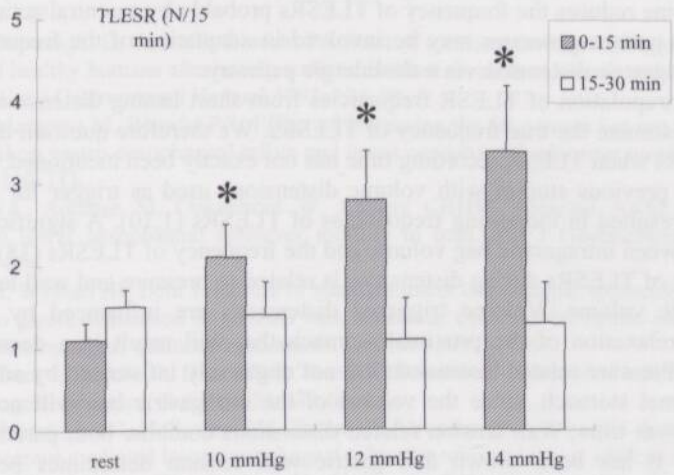
Basal LES pressure was 19±1mmHg. LES pressure increased during the gastric distensions. When the intragastric bag was deflated LES pressure decreased again to basal pressure. During gastric distensions the LES pressure was 23±2mmHg, which is significantly (p=0.02) higher than during the recovery period (19±2mmHg). At intragastric pressures of 12mmHg and 14mmHg, but not at 10mmHg, LES pressure was significantly (p<0.05) higher compared to the recovery period. LES pressure during gastric distension at 10, 12 and 14mmHg was respectively 20±2mmHg, 24±2mmHg and 25±3mmHg.

Transient lower esophageal sphincter relaxation

The frequency of TLESRs in the recovery periods was 2.6±0.4 TLESR/30min. Gastric distensions significantly (p<0.05) increased the frequency of TLESRs at 10 mmHg to 3.5±0.6 TLESR/30min, at 12mmHg to 3.8±0.7 TLESR/30min, at 14mmHg to 4.6±1.0 TLESR/30min. The frequency of TLESRs was significantly (p<0.001) higher in the first 15 min of the distension period compared to the second 15min period of distension (Figure 1). The frequency of TLESRs during 30min gastric distensions was significantly (p<0.01) correlated

with pressure (r=0.47) and wall tension (r=0.48), but not with volume. The duration of TLESRs was not significantly different between the control period and gastric distensions (control: 18±1sec; 10mmHg:17±2sec; 12mmHg: 17±2sec; 14mmHg: 18±1sec).

Figure 1. *Frequency of transient lower esophageal sphincter relaxations during intermittent isobaric distension periods - first 15min period versus second 15min period. Asterisks denote significant (p< 0,05) differences in TLESR frequency in the first 15min period compared to control period and compared to the second 15min period.*



DISCUSSION

The results of the present study support previous findings that in humans simultaneous assessment of proximal gastric pressure and lower esophageal sphincter pressure is well feasible and that gastric distension significantly increases the frequency of TLESRs (1,17-19).

During gastric distension periods the frequency of TLESRs doubled compared to rest periods. These findings correspond with the results found in previous studies in humans (1,17-19) and dogs (20). However, the frequency of TLESRs during gastric distension found by Boulant et al. (18) was considerably higher (9.1±4.0 TLESR/30min). These authors studied TLESRs at much higher intragastric pressures by reaching 75% of the pressure threshold for pain. Because of the higher pressure level the intragastric volumes were therefore larger compared to the present study: around 800ml versus at maximum 500ml in our study.

The frequency of TLESRs during distension diminished over time (second versus first 15min period). Identical patterns of a diminishing frequency of TLESRs over time are

observed after ingestion of a meal (4,5,7) and after insufflation of air (11). After meal ingestion the increase in TLESR frequency is most pronounced in the first and second postprandial hour. The TLESR frequency after intragastric air is highest in the first fifteen minutes after insufflation, thereafter it gradually returns to basal values. These observations can be explained by the fact that the stimulus diminishes over time, since the meal or air is emptied from the stomach. In the present study the stimulus used for triggering TLESR was a constant intragastric pressure. Therefore, the lower frequency of TLESRs during the second quarter suggests adaptation to the stimulus. The mechanism of triggering TLESR involves a neural pathway linking receptors in the esophagogastric region via the vagus nerve to nuclei of the brain stem. A central pattern generator in the brain stem is believed to mediate TLESR (2). Atropine reduces the frequency of TLESRs probably by a central action in the brain stem (19). This pattern generator may be involved in adaptation of the frequency of TLESRs to prolonged gastric distension via a cholinergic pathway.

Extrapolation of TLESR frequencies from short lasting distensions to 60min periods will overestimate the true frequency of TLESRs. We therefore question the results of studies on TLESRs when TLESR recording time has not exactly been mentioned.

In previous studies with volume distensions used as trigger for TLESR, increasing volumes resulted in increasing frequencies of TLESRs (1,10). A significant correlation was found between intragastric bag volume and the frequency of TLESRs (18). We found that the frequency of TLESRs during distensions is related to pressure and wall tension rather than to intragastric volume. Volume triggered distensions are influenced by the mechanism of adaptive relaxation of the proximal stomach that will result in a decrease in intragastric pressure. Pressure related distensions are not negatively influenced by adaptive relaxation of the proximal stomach, since the volume of the intragastric bag will not decrease but will increase over time. Wall tension related distensions combine both parameters: pressure and volume. It has been shown that gastric wall tension determines perception of gastric distension (16). Based on our results wall tension triggered gastric distensions (Tensostat) may be a more accurate trigger for TLESR than pressure or volume.

Distension of the proximal stomach caused a significant increase in LES pressure. The increase in LES pressure was a true increase in pressure and not an artifact caused by pressure of the intragastric bag on the sleeve pressure sensor because LES pressure is calculated relative to intragastric pressure. Other studies using either a barostat or a balloon, also observed an increase in LES pressure during gastric distension (1,18). This increase in LES pressure during gastric distension has formerly been referred to as reflex contraction and may prevent gastro-esophageal reflux. However, earlier studies have described a negative correlation between LES pressure and fundic pressure after air insufflation and have debated the reflex contraction (21).

In conclusion, acute standardized pressure driven gastric distension significantly increases the frequency of TLESRs but adaptation occurs already after 15min. The frequency of TLESR during distension is related to pressure and wall tension rather than to intragastric volume.

REFERENCES

1. Holloway RH, Hongo M, Berger K, McCallum RW. Gastric distension: a mechanism for postprandial gastroesophageal reflux. Gastroenterology 1985;89:779-784.
2. Mittal RK, Holloway RH, Penagini R, Blackshaw LA, Dent J. Transient lower esophageal sphincter relaxation. Gastroenterology 1995;109:601-610.
3. Dent J, Holloway RH, Toouli J, Dodds WJ. Mechanisms of lower oesophageal sphincter incompetence in patients with symptomatic gastrooesophageal reflux. Gut 1988; 29:1020-1028.
4. Holloway RH, Kocyan P, Dent J. Provocation of transient lower esophageal sphincter relaxations by meals in patients with symptomatic gastroesophageal reflux. Dig Dis Sci 1991;36:1034-1039.
5. Penagini R, Bartesaghi B, Conte D, Bianchi PA. Rate of transient lower esophageal sphincter relaxations of healthy humans after eating a mixed nutrient meal: time course and comparison with fasting. Eur J Gastroenterol Hepatol 1992;4:35-38.
6. Penagini R, Mangano M, Bianchi PA. Effect of increasing the fat content but not the energy load of a meal on gastro-oesophageal reflux and lower oesophageal sphincter motor function. Gut 1998;42:330-333.
7. Straathof JWA, Adamse M, Onkenhout W, Lamers CBHW, Masclee AAM. Effect of L-arginine on lower oesophageal sphincter motility in man. Eur J Gastroenterol Hepatol 2000;12:419-424.
8. Holloway RH, Wyman JB, Dent J. Failure of transient lower oesophageal sphincter relaxation in response to gastric distension in patients with achalasia: evidence for neural mediation of transient lower esophageal sphincter relaxations. Gut 1989;30:762-767.
9. Wyman JB, Dent J, Heddle R, Dodds WJ, Toouli J, Downton J. Control of belching by the lower oesophageal sphincter. Gut 1990;31:639-646.
10. Mittal RK, Chiareli, Liu J, Holloway RH, Dixon W. Atropine inhibits gastric distension and pharyngeal receptor mediated lower oesophageal sphincter relaxation. Gut 1997;41:285-290.
11. Boeckxstaens GE, Hirsch DP, Fakhry N, Holloway RH, D'Amato M, Tytgat GNJ. Involvement of cholecystokinin-A receptors in transient lower esophageal sphincter relaxations triggered by gastric distension. Am J Gastroenterol 1998;93:1823-1828.
12. Azpiroz F, Malagelada JR. Gastric tone measured by an electronic barostat in health and postsurgical gastroparesis. Gastroenterology 1987;92:934-943.
13. Whitehead WE, Delvaux M. Standardization of barostat procedures for testing smooth muscle tone and sensory thresholds in the gastrointestinal tract. Dig Dis Sci 1997;42:223-241.
14. Straathof JWA, Mearadji B, Lamers CBHW, Masclee AAM. Effect of CCK on proximal gastric motor function in humans. Am J Physiology 1998;274:G939-G944.
15. Holloway RH, Penagini R, Ireland AC. Criteria for objective definition of transient lower esophageal sphincter relaxation. Am J Physiol 1995;268:G128-G133.
16. Distrutti E, Azpiroz F, Soldevilla A, Malagelada JR. Gastric wall tension determines perception of gastric distension. Gastroenterology 1999;116:1035-1042.
17. Zerbib F, Bruley des Varannes S, Roze DC, Galmiche JP. Simultaneous study of tones of the lower esophageal sphincter and proximal stomach in healthy humans. Gastroenterol Clin Biol 1996;20(12):1078-1083.
18. Boulant J, Mathieu S, D'Amato M, Abergel A, Dapoigny M, Bommelaer G. Cholecystokinin in transient lower esophageal sphincter relaxation due to gastric distension in humans. Gut 1997;40:575-581.
19. Lidums I, Hebbard GS, Holloway RH. Effect of atropine on proximal gastric motor function and sensory function in normal subjects. Gut 2000;47:30-36.

20. Boulant J, Fioramonti J, Dapoigny M, Bommelaer G, Bueno L. Cholecystokinin and nitric oxide in transient lower esophageal sphincter relaxation to gastric distension in dogs. Gastroenterology 1994;107:1059-1066.
21. Müller-Lissner SA, Blum AL. Fundic pressure rise lowers lower esophageal sphincter pressure in man. Hepatogastroenterol 1982;28:151-152.

5

EFFECT OF GASTRIN-17 ON LOWER ESOPHAGEAL SPHINCTER CHARACTERISTICS IN MAN

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Dig Dis Sci 1997;42:2547-2551

ABSTRACT

Background: We studied the effect of gastrin-17 on lower esophageal sphincter (LES) characteristics in man.

Methods: Nine healthy volunteers participated in two experiments performed in random order during continuous infusion of saline (control) or gastrin-17 (15 pmol/kg/h). LES pressure (LESP) and transient lower esophageal sphincter relaxations (TLESR), as most important reflux mechanism, were measured with intraesophageal sleeve manometry combined with pH metry.

Results: Infusion of gastrin-17 resulted in plasma gastrin levels comparable to those reached after a mixed meal. During continuous gastrin infusion LESP decreased significantly ($P<0.05$) compared to control. The rate and duration of TLESR was not influenced by gastrin-17. Gastroesophageal reflux and the number of TLESR associated with reflux were significantly ($P<0.05$) increased during gastrin infusion.

Conclusions: These results suggest that in humans gastrin at physiological postprandial plasma concentrations decreases LESP, does not influence TLESR, but increases the percentage of TLESR associated with reflux.

INTRODUCTION

The role of gastrin in the occurrence of gastroesophageal reflux is still contradictory. On the one hand gastrin may increase reflux by stimulating acid secretion whereas on the other hand reflux of acidic gastric content might be prevented because previous studies indicate that gastrin increases lower esophageal sphincter pressure (LESP) (1-7). An intravenous bolus injection of gastrin or continuous infusion of gastrin in supraphysiological doses results in an increase in LESP (1,4). However, when gastrin is administered in doses similar to those reached seen after ingestion of a protein meal, no effect on LESP is observed or LESP even decreases (2,3,5). These results are not conclusive about the effect of gastrin on LESP under physiological conditions.

Transient lower esophageal sphincter relaxations (TLESR) are nowadays recognized as the main mechanism underlying gastroesophageal reflux (GER), both in healthy subjects and in patients with GER disease (8). The neural and hormonal mechanisms controlling TLESR are poorly understood (9). Ingestion of a meal increases the frequency of TLESR (10,11) and stimulated gastrin release. It is not known whether gastrin influences the frequency of TLESR because the effect of gastrin on TLESR has not been investigated previously.

The present study was conducted to investigate the effect gastrin, through i.v. infusion to plasma gastrin levels in physiological postprandial range, on LES characteristics including LESP and TLESR and acid reflux in nine healthy volunteers.

METHODS

Subjects

Nine healthy volunteers (five males, four females; age 22 - 29 years) participated in the study. None of the subjects had a history of gastro-intestinal disease or surgery or other illness or was on chronic medication. Informed consent was obtained from each individual. The study had been approved by the Ethics Committee of the Leiden University Medical Center.

Manometric and pH technique

The manometry catheter consisted of a multilumen polyvinyl tube (outer diameter 5.0 mm) with seven side holes and a 6 cm long sleeve sensor (Dentsleeve Pty Ltd, Belair, South Australia). Side holes are at 27, 18, 12, 9, 6, 3 and -3 cm from the center of the sleeve. The catheter was continuously perfused with gas free distilled water by a low compliance pneumohydraulic capillary infusion system (Arndorfer Medical Specialties, Greendale, Wisconsin, U.S.A.) with a rate of 0.5 ml/min. The external pressures transducers (Medex Inc., Ohio, U.S.A.) were connected via a eight channel analogue/digital converter (PC Polygraph HR, Synectics Medical, Stockholm, Sweden) to a personal computer system. The data were displayed continuously on a monitor and stored on the personal computer system (Polygram Upper GI 6.30, Gastrosoft Inc., Synectics Medical, Stockholm, Sweden).

The manometry catheter was introduced through the nose into the esophagus. After identification of LES and upper esophageal sphincter (UES) the catheter was positioned so that the sleeve sensor covered the esophagogastric junction by centering the high-pressure zone on the sleeve sensor. The proximal side hole was positioned in the pharynx, proximal of the UES and was used for identification of swallow signals. The middle side holes registered esophageal body motility. The distal side hole was used as reference for intragastric pressure. A second catheter, a glass pH electrode (Ingold LOT 440 continue glassreference electrode; Ingold Messtechnik AG, Urdorf, Germany) was passed through the nose and positioned 5 cm above the upper margin of the LES. The pH electrode had been calibrated at pH 4.0 and pH 7.0.

Study protocol

Each subject participated in two tests performed in random order on separate days with an interval of at least seven days. The subjects were fasting since 10.00 p.m the night before the test. The experiments were started at 8.30 a.m. The subjects were studied in the upright position, sitting in a comfortable chair. The manometry and pH catheter were introduced into the esophagus and positioned as described above.

Esophageal pH and motility were registered simultaneously for one hour under basal, fasting conditions (time -60 to 0 min) followed by three hours (time 0 to 180 min) during infusion of gastrin-17 or saline (control).

Two intravenous cannulas were inserted into the antecubital vein of each arm, one for intravenous infusion, the other for blood sampling. The synthetic unsulfated heptadecapeptide of human gastrin (gastrin-17-I) was continuously infused intravenously in a dose of 15 pmol/kg/h for 180 min. This dose was chosen because plasma gastrin levels are reached in the postprandial range. In the control experiment saline (NaCl 0,9 %) was administered intravenously. Blood samples for determination of plasma gastrin were taken from -60 min to

180 min at 15 min intervals. Plasma gastrin concentration was determined by radioimmunoassay as described previously (12).

To confirm that plasma gastrin levels reached during gastrin infusion were in the postprandial range, a third experiment was performed on a separate day to determine postprandial plasma gastrin levels. After an overnight fast one intravenous cannula was inserted in an antecubital vein of one arm for blood sampling. A liquid mixed meal, containing 400 ml, was drunk within 10 min (Nutridrink, Nutricia, Zoetermeer, The Netherlands; 100 ml Nutridrink contains 6.5 g fat, 17.9 g carbohydrates and 5.0 g protein, 150 kcal = 630 kJ, osmolality: 390 mOsmol/l). At regular intervals blood samples were taken for determination of plasma gastrin (-15, 0, 30, 60, 90, 120, 150, 180 min).

Lower esophageal sphincter data analysis

Lower esophageal sphincter tracings were analyzed for LES resting pressure (LESP) and LES relaxations (LESR). LESP was defined as mean end-expiratory LESP relative above intragastric pressure over a 2 min period. LESR are divided in swallow induced LESR and spontaneous LESR. Swallow induced LESR are preceded by active swallows starting with a pharyngeal contraction. Spontaneous LESR, also known as transient LES relaxation (TLESR) are divided in non-swallow related TLESR, and swallow related TLESR. Spontaneous, non swallow related TLESR are defined as decreases in LES pressure of ≥ 5 mmHg with a rate of ≥ 1 mmHg/sec, within 10 sec reaching a pressure of ≤ 2 mmHg above intragastric pressure. No swallow signal occurs in the interval from 4 sec before to 2 sec after onset of LESR. Swallow related TLESR are defined as spontaneous TLESR, irrespective of the timing of LESR to swallowing and the duration of LESR is ≥ 10 sec (9).

pH analysis data analysis

Gastroesophageal reflux episodes are defined as a sudden fall of pH below 4.0 with a duration of at least 4 sec. The number and duration of reflux episodes were counted.
The mechanisms of each reflux episode were scored using the following criteria. GER occurred during:
(1) TLESR (spontaneous LESR meeting the earlier mentioned criteria; swallow related LESR with the duration of LESR ≥ 10 sec)
(2) Swallow induced LESR (primary peristalsis or failed primary peristalsis with the duration of LESR ≤ 10 sec or multiple swallowing).
(3) LES pressure drift (a gradual loss of basal LES pressure).
(4) Absent LES pressure (LESP less than 2 mmHg above intragastric pressure).
(5) Abdominal strain (an increase in abdominal pressure).

Statistical analysis

Pressure data are expressed as mean values ± SEM. Data were analyzed for statistical significance using multiple analysis of variance (MANOVA). When this indicated a probability of less than 0.05 for the null hypothesis, Student-Newman-Keuls analyses were performed to determine which values between or within the experiments differed significantly. A P value of <0.05 was considered significant for all analyses.

RESULTS

Plasma gastrin

Ingestion of a mixed liquid meal resulted in a significant postprandial increase of plasma gastrin from 30 ± 4 (basal) to 82 ± 15 ng/l at 60 min postprandial. Three hours after ingestion plasma gastrin was 58 ± 5 ng/l (Figure 1a).

In the study for evaluation of the effect of intravenous gastrin infusion, basal plasma gastrin concentrations were not significantly different among the two experiments: 30 ± 15 ng/l (saline) and 33 ± 17 ng/l (gastrin). Infusion of 15 pmol/kg/h gastrin resulted in significant ($P<0.05$) increases in plasma gastrin over basal level and compared to the control experiment from 15 min after start of the intravenous infusion (Figure 1b).

Lower esophageal sphincter pressure

LESP in the basal period (-60 to 0 min) was not significantly different between the two experiments. During infusion of saline no significant alterations in the LESP were observed. Gastrin infusion significantly ($P<0.05$) decreased LESP from 45 to 180 min compared to the control experiment (Figure 2).

Figure 2. *Lower esophageal sphincter pressure (LESP; mmHg; mean ± SEM) during continuous infusion of saline (control) or gastrin-17 at a rate of 15 pmol/kg/h in nine healthy volunteers. Small squares represent the control experiment, closed rhomboids represent the intravenous infusion of gastrin. Asterisks denote significant (P<0.05) differences compared to control.*

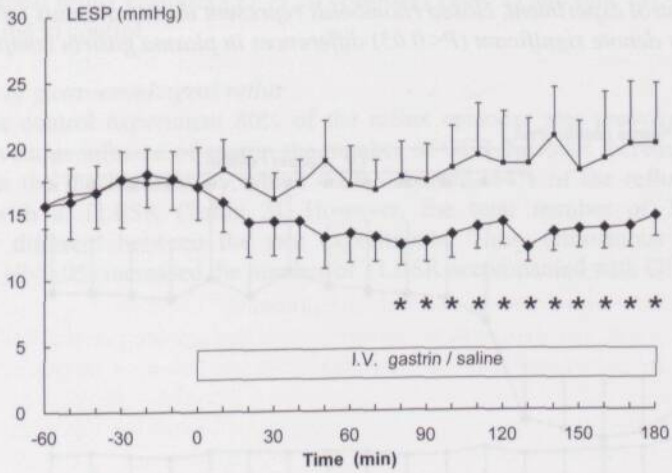


Figure 1a. *Plasma gastrin levels (ng/l; mean ± SEM) after ingestion of a 400 ml meal (100 ml contains 6.5 g fat, 17.9 g carbohydrates and 5.0 g protein) in nine healthy volunteers. Asterisks denote significant (P<0.05) differences in plasma gastrin compared to basal fasting levels.*

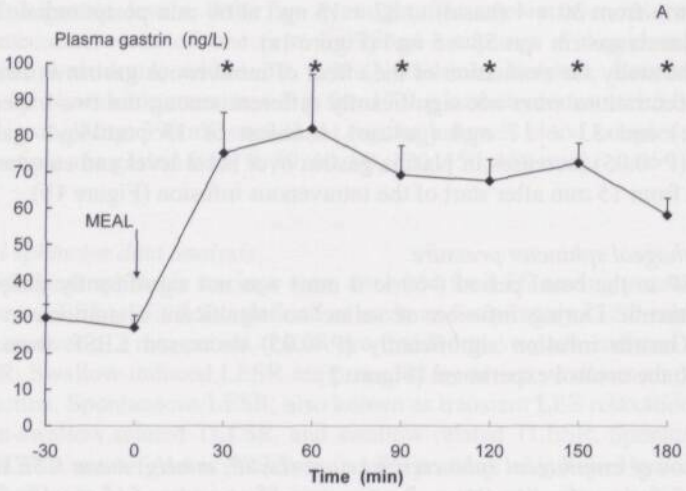
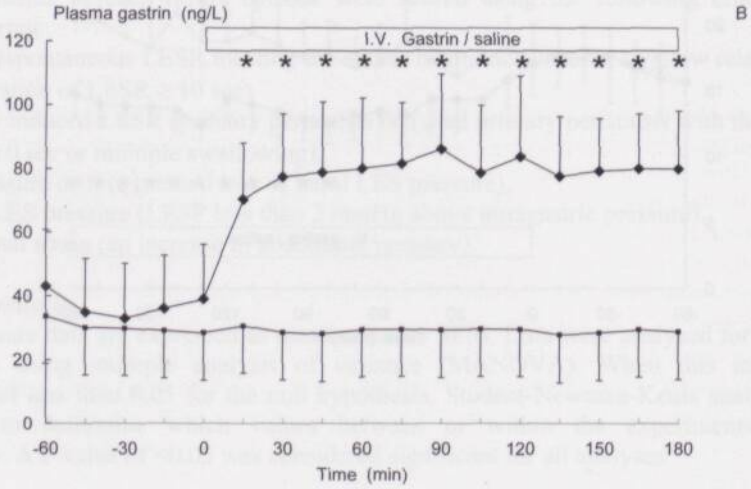


Figure 1b. *Plasma gastrin levels (ng/l; mean ± SEM) during continuous infusion of saline (control) or gastrin-17 at a rate of 15 pmol/kg/h in nine healthy volunteers. Small squares represent the control experiment, closed rhomboids represent the intravenous infusion of gastrin. Asterisks denote significant (P<0.05) differences in plasma gastrin compared to control.*



Transient lower esophageal sphincter relaxation

The mean frequency of TLESR in the basal state was 3.0 ± 0.9 per hour. Infusion of gastrin did not significantly alter the frequency of TLESR (Table 1). The duration of TLESR did not significantly change during three hours of gastrin infusion.

Table 1. *Frequency and duration of TLESR 1 hour before (fasting) and during 3 hours of intravenous infusion of saline (control) or gastrin-17 at a rate of 15 pmol/kg/h in 9 healthy subjects.*

TLESR	Control		Gastrin	
	Frequency (N/h)	Duration (sec)	Frequency (N/h)	Duration (sec)
Fasting	3.0 ± 0.9	14.9 ± 1.8	3.0 ± 0.7	14.9 ± 1.0
Infusion				
I	2.3 ± 0.5	15.7 ± 1.0	3.5 ± 1.0	13.0 ± 1.0
II	3.2 ± 0.7	17.1 ± 1.8	2.7 ± 0.5	17.3 ± 1.9
III	3.2 ± 0.6	17.3 ± 0.9	2.6 ± 0.8	16.6 ± 1.7

Gastroesophageal reflux

Acid exposure to the esophagus was of short duration in this group of healthy subjects. In the control experiment the percentage of time pH < 4 was 0.40% (range 0 - 0.75%). Intravenous infusion of gastrin resulted in significant ($P<0.05$) increases in gastroesophageal reflux episodes (55 vs 20 episodes; Table 2) and time pH<4 (2.9%, range 0 - 6.2) compared to control.

Mechanisms of gastroesophageal reflux

In the control experiment 80% of the reflux episodes was provoked by a TLESR. During intravenous infusion of gastrin the number of GER episodes increased but there was no change in the mechanisms by which GER occurred, 84% of the reflux episodes were associated with a TLESR (Table 2). However, the total number of TLESR was not significantly different between the two experiments. Thus, intravenous gastrin infusion significantly ($P<0.05$) increased the number of TLESR accompanied with GER. (Table 3)

Table 2. *Mechanisms of gastroesophageal reflux during 3 hours of intravenous infusion of either saline (control) or gastrin-17 at a rate of 15 pmol/kg/h in 9 healthy subjects. The asterisk denotes, a significant (P<0.05) difference compared to control.*

Mechanism of GER	Control	Gastrin
TLESR	16 (80%)	46 (84%)
Swallow induced LESR	1 (5%)	8 (14%)
LES pressure drift	2 (10%)	0 (0%)
Absent LES pressure	0 (0%)	0 (0%)
Abdominal strain	1 (5%)	1 (2%)
Total reflux episodes	20 (100%)	55* (100%)

Table 3. *Total number of TLESR and the number of TLESR accompanied with GER during 3 hours of intravenous infusion of saline (control) or gastrin-17 at a rate of 15 pmol/kg/h in nine healthy volunteers. Asterisks denote a significant (P<0.05) difference compared to control.*

	Control	Gastrin
TLESR	79 (100%)	75 (100%)
TLESR with pH<4	16 (20%)	46 (61%)*
Total reflux episodes	20	55*

DISCUSSION

Lower esophageal sphincter pressure is influenced by neural and hormonal mechanisms. Various gastro-intestinal polypeptides affect LES resting tone. Cholecystokinin, glucagon and secretin decrease LESP, while motilin and gastrin are supposed to increase LESP (13). Gastrin is thought to be a stimulator of the LES by increasing LESP. Intravenous injection of the synthetic analogue pentagastrin in pharmacological doses increases LESP (1), but in physiological postprandial doses gastrin does not affect LESP or even decreases LESP (2). Walker et al (2) have reported about a biphasic LES response to pentagastrin. At first, continuous infusion of pentagastrin caused a contraction of the LES but secondly at higher pentagastrin infusion rates LESP transiently decreased. In normal subjects gastrin-17 increases LESP, when given as a rapid intravenous injection. But, continuous infusion of gastrin-17 leading to serum gastrin concentrations in the physiological range did not result in significant changes in LESP (5). Others have observed a significant increase in LESP during continuous administration of gastrin-17 (3,6). We observed a small but significant decrease in LESP in response to gastrin infusion.

How do results from our study relate to those of other studies reporting no effect or even significant increments in LESP in response to gastrin infusion? First, differences in the doses of gastrin infused are probably relevant. Second, in all previous studies LESP was measured either continuously with side-hole manometry or intermittently measured using a pull-through technique. These methods are not well suited for continuous measurements of

the LES. Because the position of the side hole catheter is changing during respiration and swallowing, continuous measurement of LES resting pressure by single side hole manometry is not reliable. Pull through manometry may cause mechanical triggering and is not sufficient for identification of TLESR. Continuous registration of LES motility is best performed using a sleeve device that enables continuous registration of LES pressure and of LES relaxations including TLESR (14).

Ingestion of a meal (11) increases the number of TLESR. Food is also a stimulus for endogenous gastrin release. In the present study gastrin reaching postprandial levels did not affect the frequency nor the duration of TLESR. These results suggest that gastrin at physiological postprandial concentrations is not involved in the postprandial increase in TLESR.

TLESR is a neural reflex that is mediated through the brain stem. It is not clear whether TLESR are under hormonal control (9). The influence of other gastro-intestinal polypeptides on TLESR has not been investigated in humans except for cholecystokinin. Intravenous CCK-33 did not affect the frequency of TLESR under fasting conditions (15). However, CCK may increase the frequency of postprandial TLESR and CCK receptor antagonists possibly decrease TLESR (16,17).

Intravenous gastrin resulted in an increase of gastroesophageal reflux (GER) in healthy subjects. TLESR are the major motor mechanism by which GER occurs. Gastric distension by a meal or by intragastric air increases the number of TLESR (18). The number of TLESR was not affected by intravenous infusion of gastrin-17. The increase in GER is explained by an increase in the percentage of TLESR with acid reflux resulting from an increased gastric acid load due to gastrin infusion.

Do patients with elevated gastrin levels as in Zollinger Ellison syndrome (ZES) or pernicious anemia have abnormal LESP? Patients with elevated plasma gastrin levels due to ZES were studied by Strader et al (19). Esophageal manometry revealed normal LESP in 88% of these patients. No significant correlation was found between fasting plasma gastrin levels and the LESP. On the other hand, over 70% of patients had moderate to severe GER symptoms. Since LESP is not reduced in ZES patients the increased gastroesophageal acid reflux will result predominantly from reflux induced by TLESR. The effect of prolonged elevations in plasma gastrin levels on TLESR frequency is not known.

In conclusion, we have shown that gastrin-17 decreases LESP when infused at physiological postprandial plasma levels. Gastrin-17 did not influence the occurrence of TLESR. However, gastrin increased GER and increased the percentage of TLESR associated with reflux.

REFERENCES

1. Castell DO, Harris LD: Hormonal control of gastroesophageal-sphincter strenght. N Engl J Med 282:886-889, 1970
2. Walker CO, Frank SA, Manton J, Fordtran JS: Effect of continuous infusion of pentagastrin on lower esophageal sphincter pressure and gastric acid secretion in normal subjects. J Clin Invest 56:218-225, 1975
3. Freeland GR, Higgs RH, Castell DO, McGuigan JE: Lower esophageal sphincter and gastric acid response to intravenous infusions of synthetic human gastrin I heptadecapeptide. Gastroenterology 71:570-574, 1976
4. Henderson JM, Lidgard G, Osborne DH, Carter DC, Heading RC: Lower oesophageal sphincter response to gastrin, pharmacological or physiological? Gut 19:99-102, 1978
5. Jensen DM, McCallum RW, Corazziari E, Elashoff J, Walsh JH: Human lower esophageal sphincter responses to synthetic human gastrins 34 (G-34) and 17 (G-17). Gastroenterology 79:431-438, 1980
6. Allescher HD, Stoschus B, Wunsch E, Schusdziarra V, Classen M: Effect of human gastrin-17 with and without acid suppression on human esophageal motility. Z Gastroenterol 33:385-391, 1995
7. Lepsien G, Dietrich K: Peptone stimulation of the lower esophageal sphincter in patients with reflux disease. Z Gastroenterol 26:209-216, 1988
8. Dent J, Holloway RH, Toouli J, Dodds WJ: Mechanisms of lower oesophageal sphincter incompetence in patients with symptomatic gastrooesophageal reflux. Gut29:1020-1028,1988
9. Mittal RK, Holloway RH, Penagini R, Blackshaw LA, Dent J: Transient lower esophageal sphincter relaxation. Gastroenterology 109:601-610, 1995
10. Holloway RH, Kocyan FP, Dent J: Provocation of transient lower esophageal sphincter relaxations by meals in patients with symptomatic gastroesophageal reflux. Dig Dis Sci 36:1034-1039, 1991
11. Penagini R, Bartesaghi B, Conte D, Bianchi PA: Rate of transient lower oesophageal sphincter relaxations of healthy humans after eating a mixed nutrient meal: time course and comparison with fasting. Eur J Gastroenterol Hepat 4:35-38, 1992
12. Jansen JBMJ, Lamers CBHW: Effect of changes in serum calcium on secretin stimulated serum gastrin in patients with Zollinger-Ellison syndrome. Gastroenterology83:173-178, 1982
13. Ouyang A, Cohen S: Effects of hormones on gastrointestinal motility. Med Clin North Am 65:1111-1127, 1981
14. Dent J: A new technique for continuous sphincter pressure measurement. Gastroenterology 71:263-267, 1976
15. Ledeboer M, Masclee AAM , Batstra MR, Jansen JB , Lamers CBHW: Effect of cholecystokinin on lower oesophageal sphincter pressure and transient lower oesophageal sphincter relaxations in humans. Gut 36:39-44, 1995
16. Boulant J, Fioramonti J, Dapoigny M, Bommelaer G, Bueno L: Cholecystokinin and nitric oxide in transient lower esophageal sphincter relaxation to gastic distension in dogs. Gastroenterology 107:1059-1066, 1994
17. Clavé P, González A, Martin M, Moreno A, Cussó X, Farré A, Lluís F: Endogenous cholecystokinin (CCK) enhances postprandial gastroesophageal reflux in healthy volunteers. Gastroenterology 110:A1136, 1996
18. Holloway RH, Hongo M, Berger K, McCallum RW: Gastric distension: a mechanism for postprandial gastroesophageal reflux. Gastroenterology 89:779-784, 1985
19. Strader DB, Benjamin SB, Orbuch M, Lubensky TA, Gibril F, Weber C, Fishbeyn VA, Jensen RT, Metz DC: Esophageal function and occurence of Barrett's esophagus in Zollinger-Ellison syndrome. Digestion 56:347-356, 1995

6

EFFECT OF SOMATOSTATIN ON LOWER ESOPHAGEAL SPHINCTER CHARACTERISTICS IN MAN

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Scand J Gastroenterol 2000;35:910-915.

ABSTRACT

Background: Somatostatin (SST) is known for its inhibitory effect on the gastrointestinal tract. Transient lower esophageal sphincter relaxations (TLESR), low or absent LES pressure (LESP), swallow induced LES relaxations and are the most important reflux mechanisms.
Methods: We have studied the effect of somatostatin on lower esophageal sphincter (LES) characteristics in man. Nine healthy volunteers participated in four experiments performed in random order and double blind during continuous infusion of somatostatin (250 μg/h) or saline (control) under fasting and postprandial conditions. Esophageal motility was measured with sleeve manometry combined with pH metry.
Results: Under fasting conditions LESP and TLESR frequency were not influenced by somatostatin. Ingestion of the carbohydrate meal significantly ($p<0.01$) decreased LESP. During continuous somatostatin infusion the postprandial decrease in LESP did not occur and LESP was even significantly ($p<0.05$) increased over basal. Somatostatin did not significantly influence TLESR frequency, neither under basal conditions, nor postprandially. The residual pressure during swallow induced LES relaxation was significantly ($p<0.05$) increased by somatostatin.
Conclusions: In humans somatostatin prevents postprandial reduction in LESP, does not affect TLESRs but inhibits swallow induced LES relaxation.

INTRODUCTION

Gastroesophageal reflux (GER) is common and may occur because of low pressure at the esophagogastric junction. Recent studies in both healthy volunteers and patients with GER disease have indicated that transient lower esophageal sphincter relaxations (TLESR) are the major mechanism permitting GER (1-3). After meal ingestion not only lower esophageal sphincter pressure (LESP) decreases but also the frequency of TLESRs increases and thus may lead to a postprandial increase in reflux. Intraluminal nutrients stimulate the secretion of gastrointestinal peptides of which several are involved in regulation of motility including the LES. Gastrin and cholecystokinin (CCK) at postprandial plasma levels decrease LESP (4,5). Somatostatin (SST) is a polypeptide known for its inhibitory effect on the gastrointestinal tract (6-9). Somatostatin inhibits gastric acid secretion and may increase LESP (8,10). Somatostatin is therefore of potential clinical interest to reduce GER. However, the influence of somatostatin on reflux mechanisms, especially TLESRs, is unknown.

We have investigated the effect of somatostatin during continuous intravenous infusion on LES characteristics including LESP, TLESR and acid reflux, under both fasting and postprandial conditions.

METHODS

Subjects

Nine healthy volunteers (five females, four males; age 19 - 53 years) participated a double blind, placebo controlled randomized study. None of the subjects had a history of gastro-intestinal disease or surgery or other illness or was on chronic medication. Informed consent was obtained from each individual. The study had been approved by the Ethics Committee of the Leiden University Medical Center.

Manometric and pH technique

The manometry catheter consisted of a multilumen silicone tube (outer diameter 5.0 mm) with seven side holes located at 29, 23, 18, 13, 8, 3 and -4 cm from the mid of the 6 cm long sleeve sensor (Dentsleeve Pty Ltd, Belair, South Australia) (11). The catheter was continuously perfused with gas free distilled water by a low compliance pneumohydraulic capillary infusion system (Arndorfer Medical Specialties, Greendale, Wisconsin, U.S.A.) at a rate of 0.5 ml/min. The external pressures transducers (Medex Inc., Ohio, U.S.A.) were connected via an analogue/digital converter (PC Polygraph HR, Synectics Medical, Stockholm, Sweden) to a personal computer system. The data were displayed continuously on a monitor and stored on the personal computer system (Polygram Upper GI 6.30, Gastrosoft Inc., Synectics Medical, Stockholm, Sweden).

The manometry catheter was introduced through the nose into the esophagus and positioned so that the sleeve sensor straddled the LES. The proximal side hole was positioned in the pharynx and was used for identification of swallow signals. The middle side holes registered esophageal body motility. The distal side hole was used as reference point for intragastric pressure. A glass pH electrode (Ingold LOT 440 continue glassreference electrode; Ingold Messtechnik AG, Urdorf, Germany) was passed through the nose and positioned 5 cm above the upper margin of the LES. The pH electrode had been calibrated at pH 4.0 and pH 7.0.

Study protocol

Each subject participated in four tests performed on separate days in random order with an interval of at least seven days. Two tests were performed under fasting conditions (control vs SST) and two tests during and after ingestion of a meal (control-meal vs SST-meal). The experiments were started at 8.30 a.m. after an overnight fast. The subjects were studied in the upright position, sitting in a comfortable chair. The manometry and pH catheter were introduced into the esophagus and positioned as described above. Esophageal pH and motility were registered simultaneously for one hour under basal, fasting conditions (time -60 to 0 min) followed by three hours (time 0 to 180 min) during infusion of somatostatin or saline (control).

Two intravenous cannulas were inserted into the antecubital vein of each arm, one for intravenous infusion, the other for blood sampling. Somatostatin (Somatostatin, UCB, Brussels, Belgium) was given intravenously starting with a bolus of 250 μg followed by continuous infusion of 250 μg/h for 180 min. These doses are employed when treating patients with gastrointestinal bleeding or to reduce pancreatic exocrine secretion. In the control experiment saline (NaCl 0.9 %) was administered intravenously. In two tests a carbohydrate rich - low fat meal was given 15 min after the start of infusion. A carbohydrate

meal was chosen to avoid postprandial release of CCK, since fat and protein, but not cabohydrates, induce CCK secretion. CCK is known to influece LESP and TLESR frequency. The meal consisted of 200 g bananas blended with 125 ml water and 25 ml Roosvicee (Koninklijke De Ruiter, Baarn, The Netherlands); (2 g protein, 0 g fat and 55 g carbohydrates, 986 kJ). The subjects was asked to consume the meal within 10 min. At regular intervals (-60, 0, 30, 60, 120, 180 min) five wet swallows with 5 ml of water were given to determine swallow induced LES relaxations. Blood samples for determination of plasma hormone levels were taken from -60 min to 180 min at regular intervals (-60, -30, 0, 15, 30, 60, 90, 120, 180 min). Plasma somatostatin, CCK and gastrin concentration were determined by radioimmunoassay (12-14).

Data analysis

The tracings of esophageal motility and pH were analyzed by two investigators who were not aware of the nature of the infusion (somatostatin or placebo). The code was broken when all tracings had been analyzed.

Esophageal body

The amplitude and duration of the peristaltic waves were registered for the proximal (15 cm above the upper margin of the LES), the middle (10 cm above LES), and the distal parts (5 cm above LES) of the esophagus. The propagation velocity was also determined.

Lower esophageal sphincter

Lower esophageal sphincter tracings were analyzed for LES resting pressure (LESP) and LES relaxations (LESR). LESP was defined as mean end-expiratory LESP relative above intragastric pressure over a 2 min period. LESR are divided in swallow induced LESR and spontaneous LESR. Swallow induced LESR are preceded by active swallows starting with a pharyngeal contraction. Residual LESP after wet swallows was defined as end-expiratory nadir LESP above intragastric pressure. Spontaneous LESR, better known as transient LES relaxation (TLESR) was divided into non-swallow related TLESR, and swallow related TLESR. Spontaneous, non swallow related TLESR are defined as decreases in LESP of ≥ 5 mmHg with a rate of ≥ 1 mmHg/sec, within 10 sec reaching a pressure of ≤ 2 mmHg above intragastric pressure. No swallow signal occurs in the interval from 4 sec before to 2 sec after onset of LESR. Swallow related TLESR are defined as spontaneous TLESR, irrespective of the timing of LESR to swallowing when the duration of LESR is at least 10 sec (15).

pH analysis

Gastroesophageal reflux episodes are defined as a sudden fall of pH below 4.0 with a duration of at least 4 sec. The number and duration of reflux episodes were counted.
The mechanisms of each reflux episode were scored using the following criteria. GER occurred during:
(1) TLESR (spontaneous LESR meeting the earlier mentioned criteria; swallow related LESR with the duration of LESR ≥ 10 sec)
(2) Swallow induced LESR (primary peristalsis or failed primary peristalsis with the duration of LESR ≤ 10 sec or multiple swallowing).
(3) LES pressure drift (a gradual loss of basal LES pressure).
(4) Absent LES pressure (LESP less than 2 mmHg above intragastric pressure).
(5) Abdominal strain (an increase in abdominal pressure).

Statistical analysis

Data are expressed as mean values ± SEM. Data were analyzed for statistical significance using multiple analysis of variance (MANOVA). When this indicated a probability of less than 0.05 for the null hypothesis, Student-Newman-Keuls analyses were performed to determine which values between or within the experiments differed significantly. A p value of <0.05 was considered significant for all analyses.

RESULTS

Plasma somatostatin, CCK and gastrin

Basal plasma concentrations of somatostatin, CCK and gastrin were not significantly different between the four experiments. Ingestion of the carbohydrate meal did not influence plasma somatostatin levels. Intravenous infusion of 250 μg/h somatostatin resulted in significant (p<0.001) increases in plasma somatostatin concentrations starting from 15 min until the end of the experiment (Figure 1A).

Neither infusion of somatostatin nor ingestion of the carbohydrate meal did influence plasma CCK concentrations (Figure 1B). Ingestion of a carbohydrate meal resulted in significant (p<0.05) increases in plasma gastrin. Somatostatin significantly (p<0.05) reduced plasma gastrin levels compared to basal. During somatostatin infusion no significant changes in plasma gastrin were observed in response to the meal (Figure 1C). Gunshefski et al (10) have shown that somatostatin stimulates esophageal body motility. Our data confirms that somatostatin increases esophageal contraction amplitude and velocity.

Table 1. *Characteristics of esophageal body contractions after wet swallow in nine healty volunteers before and during continuous infusion of somatostatin. *Significant (P<0.01) increases, compared to control.*

	control	SST	control-meal	SST-meal
Amplitude (mmHg)				
Proximal	37 ± 1	53 ± 2*	40 ± 1	47 ± 2*
Mid	49 ± 2	57 ± 2*	54 ± 3	67 ± 1*
Distal	58 ± 2	65 ± 2*	55 ± 2	69 ± 2*
Duration (sec)				
Distal	3.3 ± 0.1	3.3 ± 0.1	3.1 ± 0.1	3.5 ± 0.2
Velcity (cm/sec)				
Proximal	3.3 ± 0.2	4.6 ± 0.2*	4.1 ± 0.2	5.5 ± 0.2*
Distal	3.7 ± 0.1	4.7 ± 0.2*	4.1 ± 0.1	5.2 ± 0.2*

Esophageal body motility

Characteristics of peristaltic contractions of the esophageal body are described in Table 1. The amplitude of esophageal body contractions and the velocity of peristalsis were significantly (P<0.001) increased during somatostatin infusion under both fasting and postprandial conditions.

Lower esophageal sphincter pressure

LESP in the basal period (-30 to 0 min) was not significantly different between the four experiments (control: 22 ± 4 mmHg; SST: 23 ± 4 mmHg; control-meal: 23 ± 3 mmHg; SST-meal: 23 ± 4 mmHg). During three hours of intravenous infusion of somatostatin there were no significant changes compared to the control experiment (Figure 2A). After ingestion of a meal LESP decreased significantly (p<0.01) compared to basal level from t=30 to t=75 min. During somatostatin infusion the meal did not decrease LESP but on the contrary, LESP gradually increased. The LESP was significantly (p<0.01) higher compared to the control-meal experiment from 60 to 180 min (Figure 2B). In the control experiment residual LESP after wet swallows was 9 ± 1% and 7 ± 1% after meal ingestion. Intravenous infusion of somatostatin significantly (p<0.01) increased the residual pressure after a wet swallow under postprandial (20 ± 2%) but not under fasting (11 ± 1%) conditions.

Transient lower esophageal sphincter relaxation

The frequency of TLESR was not significantly different between the basal periods of the four experiments (Table 2). During the control experiment no significant changes in TLESR frequencies were observed. Intravenous infusion of somatostatin had no significant effect on the frequency of TLESR compared to the control experiment. After ingestion of the carbohydrate meal the frequency of TLESR significantly (p<0.05) increased in the first postprandial hour (4.3 ± 0.5 TLESR/h). During somatostatin infusion the postprandial increase in TLESRs was no longer significant compared to basal.

Table 2. *Frequency of non-swallow related TLESR (number/hour; mean±SEM) one hour before (basal) and during three hours (0-180 min) of intravenous infusion of saline (control) or somatostatin (SST) at a rate of 250 μg/h in nine healthy subjects under fasting and postprandial conditions. Asterisks denote a significant (p<0.05) increase compared to basal.*

TLESR	control	SST	control-meal	SST-meal
Basal	2.1 ± 0.6	3.3 ± 0.7	2.6 ± 0.6	2.7 ± 0.6
Infusion				
0-60 min	2.1 ± 0.4	2.6 ± 0.6	4.3 ± 0.5*	3.9 ± 0.7
60-120 min	2.4 ± 0.4	3.7 ± 0.7	4.0 ± 0.4	3.0 ± 0.8
120-180 min	3.3 ± 0.6	3.4 ± 0.7	2.9 ± 0.6	4.1 ± 0.9

Mechanisms of gastroesophageal reflux

As expected, the number of reflux episodes and the percentage of time pH<4 was very low in the healthy volunteers (Table 3). In all experiments the predominant mechanism of reflux during the infusion period was TLESR.

Table 3. *Mechanisms of gastroesophageal reflux during three hours of intravenous infusion of saline (control) or somatostatin (SST) at a rate of 250 μg/h in nine healthy subjects under fasting and postprandial conditions.*

Mechanism of GER	control	SST	control-meal	SST-meal
TLESR	14 (74%)	7 (87%)	22 (88%)	14 (77%)
Swallow induced LESR	1 (6%)	0 (0%)	0 (0%)	2 (11%)
LES pressure drift	2 (10%)	0 (0%)	1 (4%)	1 (6%)
Absent LES pressure	0 (0%)	0 (0%)	1 (4%)	0 (0%)
Abdominal strain	2 (10%)	1 (13%)	1 (4%)	1 (6%)
% time pH<4	0.19%	0.17%	0.28%	0.14%

Figure 1A-C. *Plasma levels (mean ± SEM) of [A] somatostatin, [B] cholecystokinin and [C] gastrin during control (open squares) or continuous infusion of somatostatin (open diamonds). Somatostatin was given as a bolus of 250 μg followed by a continuous infusion at a rate of 250 μg/h, 15 min after start of the infusion a carbohydrate meal was ingested (closed squares and closed diamonds). Asterisks denote significant (P<0.01) differences compared to basal levels.*

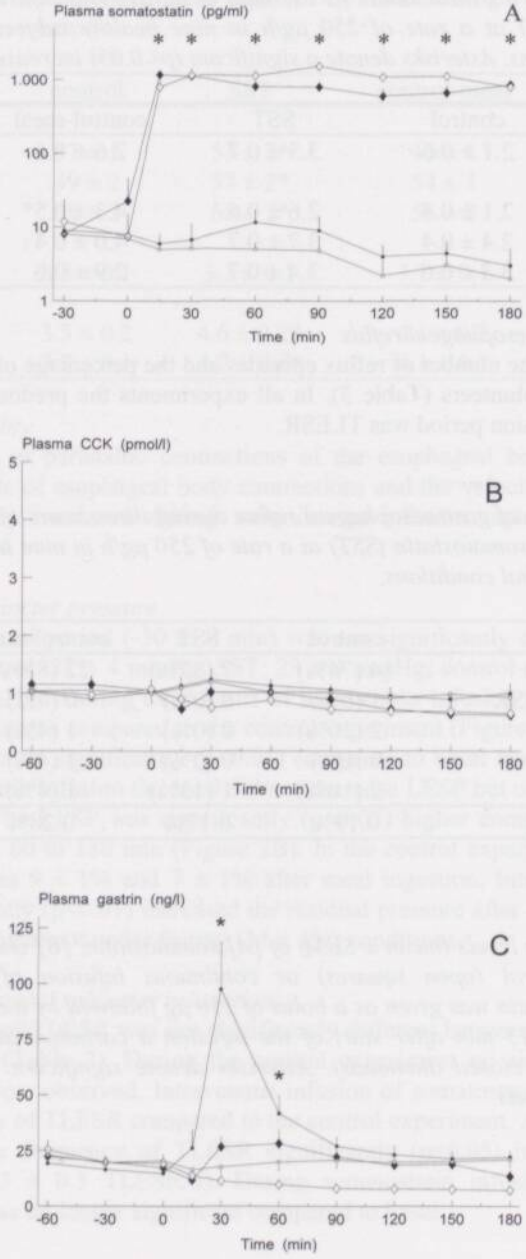
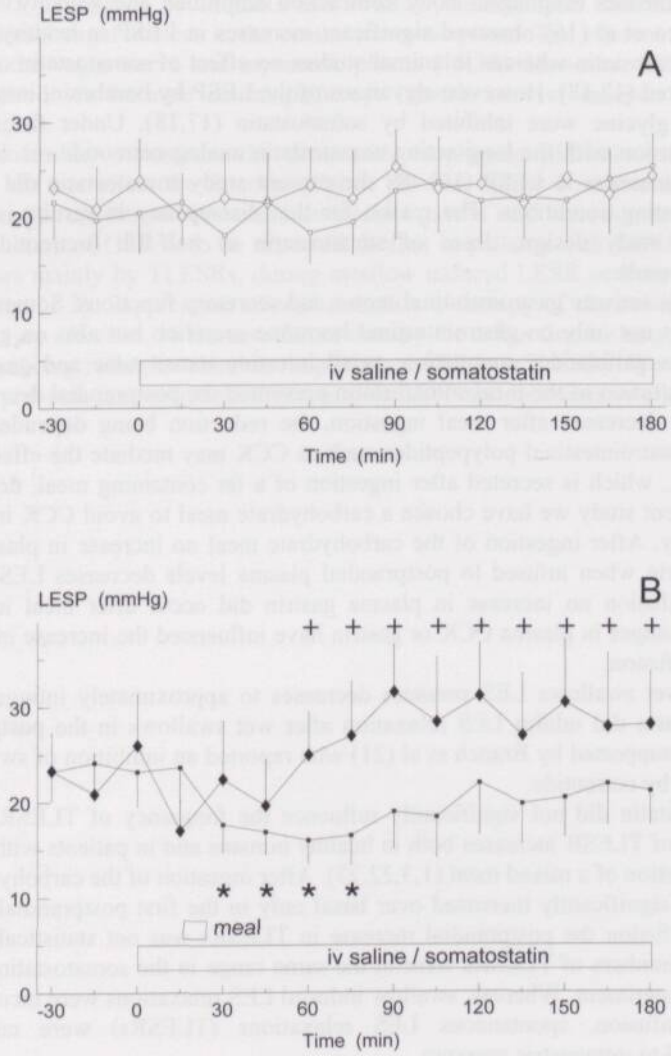

Plasma somatostatin (pg/ml)
A
1.000
100
10
1
-30
0
30
60
90
120
150
180
Time (min)
Plasma CCK (pmol/l)
B
5
4
3
2
1
0
-60
-30
0
30
60
90
120
150
180
Time (min)
Plasma gastrin (ng/l)
C
125
100
75
50
25
0
-60
-30
0
30
60
90
120
150
180
Time (min)

Figure 2A-B. *LESP (mean ± SEM) during control (open squares) or continuous intravenous infusion of 250 µg/h somatostatin (open diamonds) in nine healthy volunteers under [A] fasting and [B] postprandial conditions. The 400 ml carbohydrate rich meal consisted of 2 g protein, 0 g fat and 55 g carbohydrates (closed squares and closed diamonds). Asterisks and crosses denote significant (P<0.05) differences compared to basal LESP.*



DISCUSSION

We have shown that in healthy subjects under fasting conditions, somatostatin does not affect LESP and TLESR frequency. However, somatostatin prevents the postprandial reduction in LESP and the postprandial increase in TLESR. During somatostatin infusion swallow induced LES relaxations were incomplete, under postprandial conditions.

The effect of somatostatin on LESP has been studied previously. Gunshefski et al (10) have shown that somatostatin stimulates esophageal body motility. Our data confirms that somatostatin increases esophageal body contraction amplitude and velocity. Under fasting conditions Greco et al (16) observed significant increases in LESP in healthy volunteers in response to somatostatin whereas in animal studies no effect of somatostatin on basal LESP has been observed (17,18). However, elevations of the LESP by bombesin, intragastric alkali or intragastric glycine were inhibited by somatostatin (17,18). Under fasting conditions intravenous infusion with the long-acting somatostatin analog octreotide resulted in a small but significant increase in LESP (10). IN the present study somatostatin did not influence LESP under fasting conditions. The reason for this discrepancy in results is not obvious. Differences in study design, doses of somatostatin or half-life (octreotide) may have influenced the results.

Nutrients activate gastrointestinal motor and secretory functions. Somatostatin has an inhibitory effect not only on gastrointestinal hormone secretion but also on gastrointestinal motility such as gallbladder contraction, small intestine transit time and gastric emptying (8,19). After ingestion of the meal somatostatin prevented the postprandial decrease in LESP. Usually, LESP decreases after meal ingestion, the reduction being dependent upon meal composition. Gastrointestinal polypeptides such as CCK may mediate the effect of nutrients on LESP. CCK, which is secreted after ingestion of a fat containing meal, decreases LESP (4). In the present study we have chosen a carbohydrate meal to avoid CCK induced effects on LES motility. After ingestion of the carbohydrate meal no increase in plasma CCK was observed. Gastrin when infused to postprandial plasma levels decreases LESP (5). During somatostatin infusion no increase in plasma gastrin did occur after meal ingestion. It is unlikely that changes in plasma CCK or gastrin have influenced the increase in LESP during somatostatin infusion.

After wet swallows LES pressure decreases to approximately intragastric pressure (20). Somatostatin did inhibit LES relaxation after wet swallows in the postprandial state. This finding is supported by Branch et al (21) who reported an inhibition of swallow induced LES relaxation by octreotide.

Somatostatin did not significantly influence the frequency of TLESR when fasted. The frequency of TLESR increases both in healthy humans and in patients with symptomatic GER after ingestion of a mixed meal (1,3,22,23). After ingestion of the carbohydrate meal the frequency was significantly increased over basal only in the first postprandial hour. During somatostatin infusion the postprandial increase in TLESRs was not statistically significant. However, the numbers of TLESRs were in the same range in the somatostatin-meal and the control-meal experiment. Whereas, swallow induced LES relaxations were incomplete during somatostatin infusion, spontaneous LES relaxations (TLESRs) were complete, with relaxation equal to intragastric pressure.

The mechanism by which somatostatin influences the LES is largely unknown. Somatostatin is widely distributed in the human body both in the central nervous system and

the gastrointestinal tract. However, the esophagus has a pattern of innervation by peptide-containing neurons that is different from the stomach and intestine. Somatostatin immunoreactive nerve fibers have been found in the human LES region but are scarce (24-27). It is not clear whether the effect of somatostatin on the LES is mediated directly, via receptors or by central or peripheral neural pathways (28).

The inhibition of the LES relaxation induced by swallows but not the LES relaxation during a TLESR by somatostatin might be explained by different pathways of stimulation. It has been suggested that TLESRs share a final common pathway with swallow induced LES relaxation (29). Triggering of TLESRs and swallow induced LES relaxations is mediated through the dorsal vagal nucleus in the central nervous system. The efferent stimuli reach the LES through the vagus nerve and myenteric plexus (15,30). However, LES relaxation can also be triggered via intrinsic neural pathways (gastric nerves) independent of extrinsic nerves (15,31,32).

In the healthy volunteers the time with esophageal pH below 4 was minimal. Somatostatin did not affect the fraction of time pH was below 4 neither under fasting nor under postprandial conditions. Somatostatin inhibits gastric acid secretion and when reflux is present a reduction of GER is to be expected. Reflux in patients with GERD and in healthy subjects occurs mainly by TLESRs, during swallow induced LESR or over a very low LES resting pressure (1-3). Suppression of acid secretion is therapy of choice in GERD and has proven to be very effective. In the near future therapy for reflux disease may become focused more on influencing reflux mechanisms especially LESP and TLESRs. We have shown that somatostatin prevents the postprandial decrease in LESP and increases swallow induced LES nadir pressure.

REFERENCES

1. Dent J, Dodds WJ, Friedman RH, Sekiguchi T, Hogan WJ, Arndorfer RC, et al. Mechanism of gastroesophageal reflux in recumbent asymptomatic human subjects. J Clin Invest 1980;65:256-267.
2. Dent J, Holloway RH, Toouli J, Dodds WJ. Mechanisms of lower oesophageal sphincter incompetence in patients with symptomatic gastrooesophageal reflux. Gut 1988;29:1020-1028.
3. Dodds WJ, Dent J, Hogan WJ, Helm JF, Hauser R, Ganesh KP, Egide MS. Mechanisms of gastroesophageal reflux in patients with reflux esophagitis. N Engl J Med 1982;307:1547-1552.
4. Ledeboer M, Masclee AAM, Batstra MR, Jansen JBMJ, Lamers CBHW. Effect of cholecystokinin on lower oesophageal sphincter pressure and transient lower oesophageal sphincter relaxations in humans. Gut 1995;36:39-44.
5. Straathof JWA, Lamers CBHW, Masclee AAM. Effect of gastrin-17 on lower esophageal sphincter characteristics in man. Dig Dis Sci 1997;42:2547-2551.
6. Peeters TL, Janssens J, Vantrappen GR. Somatostatin and the interdigestive migrating motor complex in man. Regul Pept 1983;5:209-217.
7. Krejs GJ. Physiological role of somatostatin in the digestive tract: gastric acid secretion, intestinal absorption, and motility. Scand J Gastroenterol Suppl 1986;119:47-53.
8. Shulkes A. Somatostatin: physiology and clinical applications. Baillieres Clin Endocrinol Metab 1994;8:215-236.
9. Lieverse RJ, Jansen JBMJ, Jebbink MC, Masclee AAM, Rovati LC, Lamers CBHW. Effects of somatostatin and loxiglumide on gallbladder motility. Eur J Clin Pharmacol 1995;47:489-492.
10. Gunshefski LA, Rifley WJ, Slattery DW, Schifini JJ, Hartsuck M, Little AG. Somatostatin stimulation of the normal esophagus. Am J Surg 1992;163:59-62.
11. Dent J. A new technique for continuous sphincter pressure measurement. Gastroenterology 1976;71:263-267.
12. Götz JM, Veenendaal RA, Biemond I, Muller ESM, Veselic M, Lamers CBHW. Serum gastrin and mucosal somatostatin in Helicobacter Pylori associated gastritis. Scand J Gastroenterol 1995;30:1064-1068.
13. Jansen JBMJ, Lamers CBHW. Radioimmunoassay of cholecystokinin in human tissue and plasma. Clin Chim Acta 1983;131:305-316.
14. Jansen JBMJ, Lamers CBHW. Effect of changes in serum calcium on secretin stimulated serum gastrin in patients with Zollinger-Ellison syndrome. Gastroenterology 1982;83:173-178.
15. Mittal RK, Holloway RH, Penagini R, Blackshaw LA, Dent J. Transient lower esophageal sphincter relaxation. Gastroenterology 1995;109:601-610.
16. Greco AV, Bianco A, Altomonte L, D'Acquarica L, Ghirlanda G. Effect of somatostatin on lower esophageal sphincter (LES) pressure and serum gastrin in normal and achalasic subjects. Horm Metab Res 1982;14:26-28.
17. Bybee DE, Brown FC, Georges LP, Castell DO, McGuigan JE. Somatostatin effects on lower esophageal sphincter function. Am J Physiol 1979;237:E77-E81.
18. Parkman HP, Reynolds JC. Somatostatin selectively inhibits excitatory contractile pathways of the feline lower esophageal sphincter. Regul Pept 1990;27:325-334.
19. Fisher RS, Rock E, Levin G, Malmud L. Effects of somatostatin on gallbladder emptying. Gastroenterology 1987;92:885-890.
20. Aliperti G, Clouse RE. Incomplete lower esophageal sphincter relaxation in subjects with peristalsis: prevalence and clinical outcome. Am J Gastroenterol 1991;86:609-614.

21. Branch MS, Gessner FM, Smith JW, Brazer SR. Somatostatin analogue (octreotide) inhibits lower esophageal sphincter relaxation. Gastroenterology 1991;100:A425.
22. Holloway RH, Kocyan FP, Dent J. Provocation of transient lower esophageal sphincter relaxations by meals in patients with symptomatic gastroesophageal reflux. Dig Dis Sci 1991;36:1034-1039.
23. Penagini R, Bartesaghi B, Conte D, Bianchi PA. Rate of transient lower oesophageal sphincter relaxations of healthy humans after eating a mixed nutrient meal: time course and comparison with fasting. Eur J Gastroenterol Hepat 1992;4:35-38.
24. Aggestrup S, Uddman R, Jensen SL, Hakanson R, Sundler F, Schaffalitzky de Muckadell O, et al. Regulatory peptides in lower esophageal sphincter of pig and man. Dig Dis Sci 1986;31:1370-1375.
25. Wattchow DA, Furness JB, Costa M, O'Brien PE, Peacock M. Distribution of neuropeptides in the human esophagus. Gastroenterology 1987;93:1363-1371.
26. Ferri GL, Adrian TE, Soimero L, Blank M, Cavalli D, Biliotti G, et al. Intramural distribution of immunoreactive vasoactive intestinal polypeptide (VIP), substance P, somatostatin and mammalian bombesin in the oesophago-gastro-pyloric region of the human gut. Cell Tissue Res 1989;256:191-197.
27. Singaram C, Sengupta A, Sugarbaker DJ, Goyal RK. Peptidergic innervation of the human esophageal smooth muscle. Gastroenterology 1997;101:1256-1263.
28. Fox JE. Control of gastrointestinal motility by peptides: old peptides, new tricks - new peptides, old tricks. Gastroenterol Clin North Am 1989;18:163-177.
29. Holloway RH, Wyman JB, Dent J. Failure of transient lower oesophageal sphincter relaxation in response to gastric distension in patients with achalasia: evidence for neural mediation of transient lower oesophageal sphincter relaxations. Gut 1989;30:762-767.
30. Mittal RK, Balaban DH. The esophagogastric junction. N Engl J Med 1997;336:924-932.
31. Schulze-Delrieu K, Percy WH, Ren J, Shirazi SS, Von Derau K. Evidence for inhibition of opossum LES through intrinsic gastric nerves. Am J Physiol 1989;256:G198-G205.
32. Grundy D, Scratcherd T. A permissive role for the vagus nerves in the genesis of antro-antral reflexes in the anaesthetized ferret. J Physiol (Lond) 1986;381:377-384.

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BOMBESIN INDUCED CHANGES IN LES CHARACTERISTICS IN REFLUX DISEASE AND POST-FUNDOPLICATION

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Submitted for publication

ABSTRACT

Background: In healthy controls bombesin affects gastrointestinal motility including a rise in lower esophageal sphincter (LES) pressure. Little is known on the effect of bombesin on LES pressure and reflux mechanisms such as transient lower esophageal sphincter relaxations (TLESRs) in reflux patients (GERD) with low LES pressure and patients post-fundoplication.
Methods: We studied LES characteristics (sleeve manometry) in 10 healthy controls, 22 patients with GERD and 24 patients post-fundoplication. All subjects were studied twice, in random order during intravenous bombesin or placebo. LES pressure, TLESRs and reflux were scored under based condition and after gastric air distension.
Results: Basal LES pressure was 16 ± 2 mmHg in controls, 11 ± 1 mmHg in GERD ($p<0.05$ vs controls) and 18 ± 2 mmHg post-fundoplication. Peak increments in LES pressure in response to bombesin were 20 ± 4 mmHg (controls), 17±3 mmHg (GERD) and 12±2 mmHg (post-fundoplication; $p<0.05$ vs controls and GERD). Basal TLESR frequency was significantly ($p<0.05$) reduced post-fundoplication (0.6±0.1/20min) versus controls (1.6±0.3 TLESR/20min) and GERD (1.2±0.3 TLESR/20min). Gastric distension significantly ($p<0.05$) increased TLESR to 4.0 ± 1.0 TLESR/20 min (controls); 3.4 ± 0.4 TLESR/20 min (GERD); 1.7 ± 0.5 TLESR/20 min (post-fundoplication). Bombesin did not affect TLESR frequency neither under basal conditions nor after gastric distension.
Conclusions: Bombesin significantly increases LES pressure not only in healthy subjects but also in patients with GERD and post-fundoplication. TLESR frequency is not influenced by bombesin.

INTRODUCTION

The tetradecapeptide bombesin, originally isolated from the skin of the European amphibian Bombina Bombina, is the mammalian counterpart of gastrin releasing peptide [1]. Bombesin has a wide range of biological effects that includes the release of gut hormones, stimulation of gastric and pancreatic secretion and gastrointestinal motility [2-6]. Bombesin like immunoreactivity has been demonstrated in gastrointestinal endocrine cells and in nerve fibres innervating mucosa and muscle layers [7-9]. Specific bombesin binding sites have been identified in fibres innervating both circulated and longitudinal muscle tissue [10]. Recent studies have provided evidence for a direct myogenic action of bombesin-like peptides in the gastrointestinal tract, including the esophagus [10].

With respect to the esophagus: bombesin, when given intravenously increases lower esophageal sphincter (LES) pressure in healthy subjects [1-3]. Bombesin does not act on LES pressure via cholinergic pathways nor through release of gastrointestinal hormones since the effect of bombesin is not influenced by atropine or somatostatin [3]. Because of the effect on LES pressure, bombesin-like substances deserve further evaluation in conditions with LES dysfunction such as in patients with gastroesophageal reflux disease (GERD). Reflux of acid occurs when LES pressure is low or during transient lower esophageal sphincter relaxations (TLESRs). These TLESRs have been well recognized as the most important mechanism through which reflux occurs [12-15].

Aim of the present study was to investigate the effect of bombesin on LES characteristics especially on TLESRs in healthy controls, in patients with GERD who usually

have a low LES pressure and patients after fundoplication. After fundoplication LES pressure at the esophagogastric junction increases significantly and the frequency of TLESRs is significantly reduced [16-18]. It is not known whether bombesin will influence LES characteristics in patients post-fundoplication. TLESRs were studied under basal conditions and after provocation through gastric distension with air.

METHODS

Subjects

Three groups of subjects were studied. A total of 56 subjects agreed to participate:
1) healthy volunteers (n=10; 6 females, 4 males; age 20 - 48 years). None of them had a history of gastro-intestinal disease or previously underwent surgery or was on chronic medication.
2) patients with GERD (n=22; 8 females, 14 males; age 27 - 59 years). Gastroesophageal acid reflux in these patients was documented by abnormal result during 24 hour ambulatory pH metry and/or endoscopy showing erosive reflux disease. Results of 24 hour pH metry: total time pH<4: 10.5±2.6%; (normal value <4.0%).
3) patients after antireflux surgery (n=24; 11 females, 13 males; age 31 - 76 years). Fourteen patients underwent a complete (360o) Nissen fundoplication. Ten patients underwent a partial (180°-270°) fundoplication. Postoperatively the percentage of total time with esophageal pH<4 was 3.7±0.8%. Informed consent was obtained from each individual. The study had been approved by the Ethics Committee of the Leiden University Medical Center.

Manometric and pH technique

A manometry catheter consisting of a multilumen silicone tube (outer diameter 5.0 mm) with seven side-holes located at 29, 23, 18, 13, 8, 3 and -4 cm from the mid of the 6 cm long sleeve sensor (Dentsleeve Pty Ltd, Belair, South Australia) was used. The catheter was continuously perfused with gas free distilled water by a low compliance pneumohydraulic capillary infusion system at a rate of 0.5 ml/min. The external pressure transducers (Medex Inc., Ohio, U.S.A.) were connected via an analogue/digital converter (PC Polygraph HR, Medtronic, Denmark) to a personal computer system. The data were displayed continuously on a monitor and stored on a personal computer system for later analyses.

The manometry catheter was positioned so that the sleeve sensor straddled the LES. The proximal side hole was positioned in the pharynx and was used for identification of swallow signals. The middle side-holes registered esophageal body motility. The distal side-hole was used as reference point for intragastric pressure. A glass pH electrode (Ingold LOT 440 continue glassreference electrode; Ingold Messtechnik AG, Urdorf, Germany) was passed through the nose and positioned 5 cm above the upper margin of the LES. The pH electrode had been calibrated at pH 4.0 and pH 7.0.

Study protocol

Two experiments were performed (bombesin, placebo) in random order and on separate days. The experiments were started at 9.00 a.m. after an overnight fast. Subjects were studied in the upright position, sitting in a comfortable chair. The manometry and pH catheter were introduced and positioned as described above. Infusion of bombesin or placebo

was started at time 0 min until 20 min and recording was continued for 60 min. Air was insufflated twice into the stomach in 750 ml portions through the distal intragastric side-hole of the manometry assembly with a 20 min interval. Bombesin was given i.v. a dose of 5 ng/kg/min for 20 min. When subjects had not belched or were unable to belch at the end of the experiment, the air was removed from the stomach.

Lower esophageal sphincter data analysis

Lower esophageal sphincter tracings were analyzed for LES resting pressure and TLESRs. LES pressure was defined as mean end-expiratory LES pressure relative above intragastric pressure over a 2 min period. LES pressure was scored with 10 min interval. TLESRs defined as decreases in LES pressure of ≥ 5 mmHg with a rate of ≥ 1 mmHg/sec, within 10 sec reaching a pressure of ≤ 2 mmHg above intragastric pressure. No swallow signal occurs in the interval from 4 sec before to 2 sec after onset of LES relaxation. Swallow related TLESR are defined as spontaneous TLESR, irrespective of the timing of LESR to swallowing when the duration of LESR is at least 10 sec [13, 14]. The occurrence of TLESRs was scored in three subsequent 20 min periods: 1) fasting, bombesin vs placebo i.v. and 2) twice with air insufflation, bombesin vs placebo i.v.

Gastroesophageal acid reflux

Gastroesophageal acid reflux episodes are defined as a sudden fall of pH below 4.0 with a duration of at least 4 sec. The number and duration of reflux episodes were counted. The mechanisms of each acid reflux episode were scored using previously described criteria [14].

Statistical analysis

Data are expressed as mean ± SEM. Data were analyzed for statistical significance using (multiple) analysis of variance. When this indicated a probability of less than 0.05 for the null hypothesis, Student-Newman-Keuls analyses were performed to determine which values between or within the experiments differed significantly. The Mann-Whitney U test for comparison of nonparametric data was used for statistical analysis to compare results between the groups of patients and the controls. A p value of <0.05 was considered significant for all analyses.

RESULTS

Lower esophageal sphincter pressure

Basal LES pressure was 16 ± 2 mmHg in controls, 11 ± 1 mmHg patients with GERD ($p<0.05$ vs controls) and 18 ± 2 mmHg in patients after fundoplication (Figure 1). Basal LES pressure was significantly ($p<0.05$) higher in patients after fundoplication compared to patients with GERD. Intravenous bombesin significantly ($p<0.05$ - $p<0.001$) increased LES pressure in all subjects starting from 10 min until 60 min. Peak increments in LES pressure in response to bombesin were 20 ± 4 mmHg in controls, 17 ± 3 mmHg in patients with GERD and 12 ± 2 mmHg in patients after fundoplication ($p<0.05$ vs controls and GERD).

Transient lower esophageal sphincter relaxations

In the placebo experiment, under basal conditions the frequency of TLESR was not significantly different between controls and patients with GERD (1.6 ± 0.3 vs. 1.2 ± 0.3 TLESR/20 min). Patients after fundoplication had a significantly ($p<0.05$) lower frequency of TLESR (0.6 ± 0.1 TLESR/20 min) compared to controls. (Figure 2 upper panel). Bombesin did not affect TLESR frequency.

The frequency of TLESRs increased significantly ($p<0.05$) after gastric distension in controls (to 4.0 ± 1.0 TLESR/20 min), patients with GERD (to 3.4 ± 0.4 TLESR/20 min) and patients after fundoplication (to 1.7 ± 0.5 TLESR/20 min; Figure 2, lower panel). The number of TLESRs after gastric distension in patients post-fundoplication was significantly ($p<0.05$) lower compared to controls. After infusion of bombesin, gastric distension again significantly ($p<0.05$) increased the frequency of TLESRs in controls, patients with GERD and patients after fundoplication (Figure 2, lower panel). Bombesin did not influence the effect of gastric air distension with air on the frequency of TLESRs.

Table 1. *Gastroesophageal reflux (GER) in healthy controls, patients with gastroesophageal reflux disease (GERD) and patients post-fundoplication under fasting conditions in response to placebo i.v. and in response to bombesin i.v. Results are expressed as group during the 60 min period accumulated data of each group during 60 min period.*

	Control (n=10)	GERD (n=22)	Fundoplication (n=24)
Placebo			
GER episodes (in 60 min) total	9	24	24
Time pH<4 (%)	0.4%	0.8%	0.9%
GER during TLESR (%) /GER episodes	78%	63%	71%
Bombesin			
GER episodes in 60 min (total N)	19	70	24
Time pH<4 (%)	1.4%	4.4%*	1.1%
GER during TLESR (%)/GER episodes	74%	79%	71%

Gastroesophageal acid reflux

The number of reflux episodes and the percentage of time with pH < 4 under basal conditions in response to placebo i.v. was low in healthy volunteers. This was also the case for patients with GERD and patients after fundoplication (Table 1). In patients with GERD acid reflux increased significantly ($p<0.05$) in response to bombesin infusion. The majority of gastroesophageal acid reflux occurred during TLESRs.

Figure 1. *Lower esophageal sphincter pressure (mean ± sem) in 10 healthy controls (small squares), 22 patients with gastroesophageal reflux disease (diamonds) and 24 patients with fundoplication (triangles). The upper panel shows LES pressure under fasting conditions in response to i.v. placebo.. The lower panel shows LES pressure in response to bombesin infusion.*

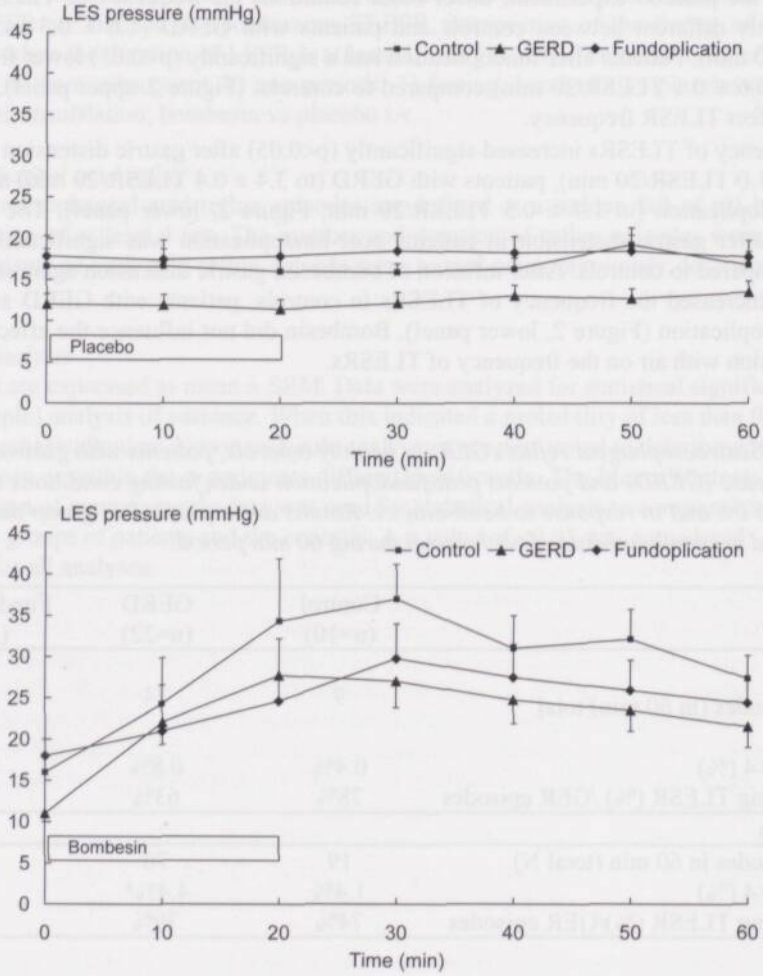
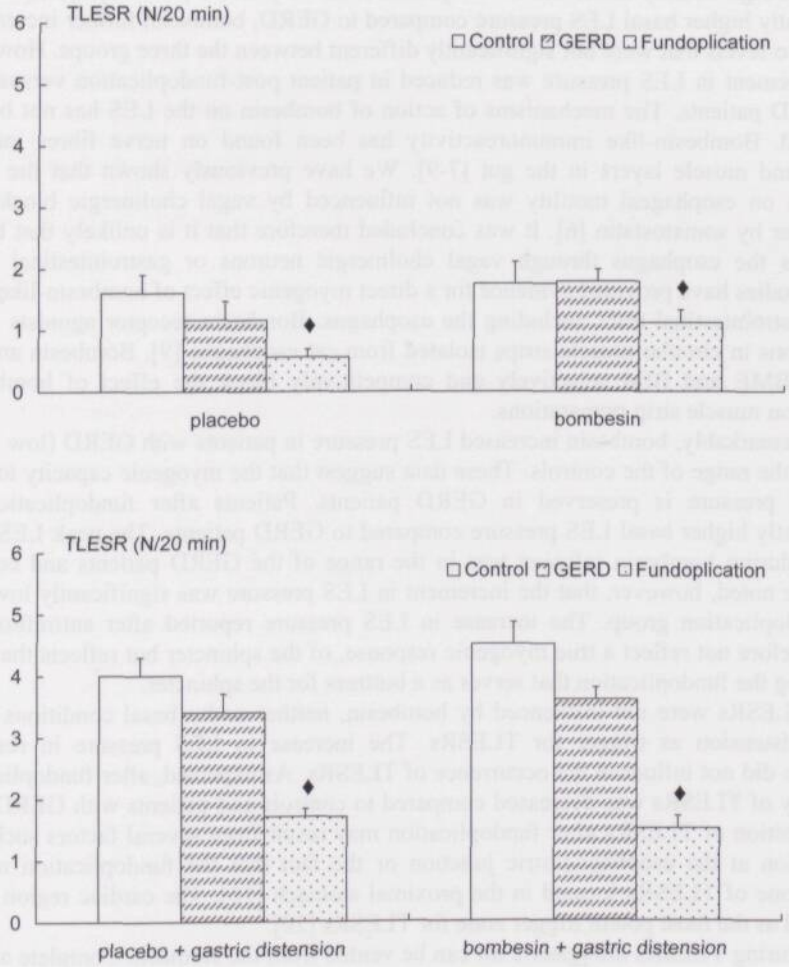


Figure 2. *Frequency of transient lower esophageal sphincter relaxations (mean ± sem/20 min) in 10 healthy controls (open bars), 22 patients with gastroesophageal reflux disease (shaded bars) and 24 patients post-fundoplication (dotted bars). The upper panel shows TLESR frequency under fasting conditions during i.v. placebo and i.v. bombesin. The lower panel shows TLESR frequency in response to gastric distension during i.v. placebo and during i.v. bombesin. Diamonds denote significant (p <0.05) differences compared to control.*



DISCUSSION

It was shown that bombesin significantly increases LES pressure in controls, GERD patients and patients post-fundoplication. Bombesin did not influence the frequency of TLESR neither under basal conditions nor after gastric air distension.

With respect to the effect of bombesin on LES pressure, our results are in line with previous observations that bombesin significantly increases LES pressure in healthy subjects [2, 6, 19]. Bombesin increased LES pressure to a same extend in the patients with GERD, who had a significantly lower basal LES pressure. In the patients post-fundoplication, with a significantly higher basal LES pressure compared to GERD, bombesin further increased LES pressure to levels that were not significantly different between the three groups. However, the peak increment in LES pressure was reduced in patient post-fundoplication versus controls and GERD patients. The mechanisms of action of bombesin on the LES has not been fully elucidated. Bombesin-like immunoreactivity has been found on nerve fibres innervating mucosa and muscle layers in the gut [7-9]. We have previously shown that the effect of bombesin on esophageal motility was not influenced by vagal cholinergic blockade with atropine or by somatostatin [6]. It was concluded therefore that it is unlikely that bombesin influences the esophagus through vagal cholinergic neurons or gastrointestinal peptides. Recent studies have provided evidence for a direct myogenic effect of bombesin-like peptides in the gastrointestinal tract, including the esophagus. Bombesin receptor agonists stimulate contractions in circular muscle strips isolated from cat esophagus [9]. Bombesin antagonists such as BME and BIM selectively and competitively block the effect of bombesin-like peptides on muscle strip preparations.

Remarkably, bombesin increased LES pressure in patients with GERD (low LESP) to levels in the range of the controls. These data suggest that the myogenic capacity to increase sphincter pressure is preserved in GERD patients. Patients after fundoplication had a significantly higher basal LES pressure compared to GERD patients. The peak LES pressure reached during bombesin infusion was in the range of the GERD patients and controls. It should be noted, however, that the increment in LES pressure was significantly lower in the post-fundoplication group. The increase in LES pressure reported after antireflux surgery may therefore not reflect a true myogenic response, of the sphincter but reflects the response to creating the fundoplication that serves as a buttress for the sphincter.

TLESRs were not influenced by bombesin, neither under basal conditions nor after gastric distension as trigger for TLESRs. The increase in LES pressure in response to bombesin did not influence the occurrence of TLESRs. As expected, after fundoplication the frequency of TLESRs was decreased compared to controls and patients with GERD [16-18]. The inhibition of TLESRs after fundoplication may result from several factors such as local denervation at the esophagogastric junction or the fact that the fundoplication masks the trigger zone of TLESRs located in the proximal stomach [16]. The cardiac region has been identified as the most potent trigger zone for TLESRs [20].

During TLESRs intragastric air can be vented from the stomach. Complete absence or a very low frequency of TLESRs may lead to symptoms as gas bloat. When LES pressure was elevated, after bombesin infusion, TLESRs occurred at the same frequency as during placebo i.v. Thus, bombesin did not negatively affect LES relaxation, neither during swallowing as shown previously, nor during TLESRs.

Despite an increase in LES pressure, acid reflux was not reduced, but even increased after bombesin infusion. Bombesin is known to stimulate gastrin release and gastric acid secretion [2, 3]. The results of the present study do not support a further exploration of the role of bombesin or bombesin agonists in reflux related disorders.

In conclusion: bombesin significantly increases LES pressure not only in healthy controls, but also in patients with GERD and patients after fundoplication. TLESR frequency is not influenced by bombesin.

REFERENCES

1. Anastasi A, Erspamer W, Baca M. Isolation and amino acid sequences of alytestin and bombesin, two analogous active tetradecapeptides from the skin of the European discoglossid frogs. Arch Biochem Biophys 1972;148:443-6.
2. Delle Fave G, Annibale B, De Magistris L, et al. Bombesin effects on human GI functions. Peptides1985;6:113-6.
3. Jansen JBMJ, Lamers CBHW. The effect of somatostatin on bombesin-stimulated serum gastrin and gastric acid secretion in man. Digestion 1981;21:193-7.
4. Jebbink MCW, Jansen JMBJ, Mooy DM, Rovati LC, Lamers CBHW. Effect of the specific cholecystokinin-receptor antagonist loxiglumide on bombesin-stimulated pancreatic enzyme secretion in man. Regul Pept 1991;32:361-8.
5. Douglas BR, Jebbink MCW, Tjon a Tham RTO, Jansen JBMJ, Lamers CBHW. The effect of loxiglumide (CR-1505) on basal and bombesin-stimulated gallbladder volume in man. Eur J Pharmacol 1989;166:307-10.
6. Masclee AAM, Lam WF, Lamers CBHW. Effect of bombesin on esophageal motility in humans. Diseases of the Esophagus 1999:12:54-9.
7. Polak JM, Bloom SR, Hobbs S. Distribution of a bombesin-like immunocreactivity in the rat gastrointestinal tract. Lancet 1976;307:1109-10.
8. Dockray GJ, Vaillant C, Walsh JH. The neuronal origin of bombesin-like immunoreactivity in the rat gastrointestinal tract. Neuroscience 1979;4:1561-8.
9. Milusheva EA, Kortezova NI, Mizhorkova ZN, Papsova M, Voy DH, Balint A, Vizi ES, Varga G. Role of different bombesin receptor subtypes mediating contractile activity in cat upper gastrointestinal tract. Peptides 1998;19:549-56.
10. Moran TH, Moody TW, Hostetler AM, Robinson PH, Goldrich M, McHugh PR. Distribution of bombesin binding sites in the gastrointestinal tract. Peptides 1988;9:643-9.
11. Dent J, Dodds WJ, Friedman RH, et al. Mechanisms of gastroesophageal reflux in recumbent asymptomatic human subjects, J Clin Invest 1980;65:256-67.
12. Dent J, Holloway RH, Toouli J, et al. Mechanism of lower oesophageal sphincter incompetence in patients with symptomatic gastrooesophageal reflux. Gut 1988;29:1020-8.
13. Mittal RK, Holloway RH, Penagini R, et al. Transient lower esophageal sphincter relaxation. Gastroenterology 1995;109:601-10.
14. Holloway RH, Penagini R, Ireland AC. Criteria for objective definition of transient lower esophageal sphincter relaxation. Am J Physiol 1995;268:G128-33.
15. Trudgill NJ, Riley SA. Transient lower esophageal sphincter relaxations are no more frequent in patients with gastroesophageal reflux disease than in asymptomatic volunteers. Am J Gastroenterol 2001;96:2569-74.
16. Ireland AC, Holloway RH, Toouli J, et al. Mechanisms underlying the antireflux action of fundoplication. Gut 1993;34: 303-8.
17. Masclee AAM, Horbach JM, Ledeboer M, et al. Prospective study of the effect of the Belsey Mark-IV fundoplication on reflux mechanisms. Scand J Gastroenterol 1998;33:905-10.
18. Straathof JWA, Ringers J, Masclee AAM. Prospective study on the effect of laparoscopic Nissen fundoplication on reflux mechanisms. Br J Surg 2001;88:1519-24.
19. Corazziari E, Delle Fave G, Pozzessere C, et al. Effect of bombesin on lower esophageal sphincter pressure in humans. Gastroenterology 1982;83:10-4.
20. Franzi SJ, Martin CJ, Cox MR, et al. Response of canine lower esophageal sphincter to gastric distension. Am J Physiol 1990:259:G380-5.

8

EFFECT OF L-ARGININE ON LOWER ESOPHAGEAL SPHINCTER MOTILITY IN MAN

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Eur J Gastroenterol Hepatol 2000;12:419-24.

ABSTRACT

Background: Inhibitory responses of the lower esophageal sphincter are mediated via an L-arginine/nitric oxide pathway. L-arginine is known as the precursor of NO. We have studied the effect of intravenous L-arginine on lower esophageal sphincter (LES) characteristics in man.

Design: Twelve healthy subjects participated in a double blind, placebo controlled randomized study.

Methods: We investigated the effect of continuous infusion of L-arginine (500mg/kg body weight/120 min) in six subjects under fasting conditions. Six other subjects were studied under postprandial conditions. LES pressure (LESP), swallow induced LES relaxations and transient lower esophageal sphincter relaxations (TLESR), were measured with sleeve manometry combined with pH metry. The meal consisted of a carbohydrate-high fat meal. Blood samples were taken before and after administration of L-arginine or saline to determine plasma levels of amino acids, cholecystokinin and gastrin.

Results: Plasma levels of arginine and citrulline significantly ($p<0.05$) increased during L-arginine infusion. L-arginine did not affect plasma hormone levels. Under fasting conditions LESP and TLESR were not influenced by L-arginine. Ingestion of the carbohydrate-high fat meal significantly decreased LESP. L-arginine did not significantly influence TLESR frequency, neither under fasting conditions, nor postprandially.

Conclusions: These results suggest that in humans under fasting or postprandial conditions intravenous infusion of L-arginine does not influence LES motility.

INTRODUCTION

Nonadrenergic noncholinergic (NANC) nerves mediate inhibitory responses in the gastrointestinal tract and regulate important physiological reflexes such as relaxation of the lower esophageal sphincter (LES) after swallowing and receptive relaxation of the proximal stomach [1-3]. Nitric oxide (NO) has been recognized as inhibitory neurotransmitter of NANC nerves in gastrointestinal smooth muscles [4]. NO is synthesized from the amino acid L-arginine by nitric oxide synthases. In the metabolic route L-arginine is the precursor of NO whereas citrulline is byproduct of the reaction. Recent studies have described the involvement of the L-arginine - nitric oxide pathway in esophageal motility. Studies in dog [5] and opossum [6,7] have shown that NO plays an important role in NANC mediated responses in the esophageal body and LES. The antagonistic action of nitric oxide synthase inhibitors is reversed by L-arginine but not by D-arginine. In vitro examination of circular smooth muscle of the human esophageal body and LES have shown similar effects [8,9].

Transient lower esophageal sphincter relaxations (TLESR) are prolonged relaxations of the LES not associated with swallowing [10]. TLESR are thought to be a venting mechanism allowing release of air from the stomach. However, during TLESR gastroesophageal acid reflux may occur [11]. In fact, in humans TLESR are the most common mechanism permitting gastroesophageal reflux. Gastric distension or ingestion of a meal are potent triggers for TLESR [12]. NO may be involved in the occurrence of TLESRs as suggested by Boulant *et al* [13] but the exact mechanism of triggering TLESR is not known.

If the availability of L-arginine is considered as a rate-limiting factor for NO production, administration of the precursor L-arginine may be expected to result in increased NO formation. The influence of intravenous L-arginine on the LES and TLESR, is unknown. We have investigated in healthy subjects the effect of intravenous administration of L-arginine on LES characteristics including LESP, TLESR and acid reflux, both under fasting and postprandial conditions.

METHODS

Subjects

In a double blind, randomized, placebo controlled study we have evaluated the effect of intravenous L-arginine on LES and esophageal motility. Twelve healthy subjects were studied twice in random order during iv infusion of saline or L-arginine. Six subjects were studied during fasting conditions (3 females, 3 males; age 18 - 26 years). Six other subjects (5 females, 1 male; age 19 - 31 years) were studied under postprandial conditions. None of the subjects had a history of gastro-intestinal disease or surgery or other illness or was on chronic medication. Informed consent was obtained from each individual. The study had been approved by the Ethics Committee of the Leiden University Medical Center.

Manometric and pH technique

The manometry catheter consisted of a multilumen silicone tube (outer diameter 5.0 mm) with seven side holes located at 29, 23, 18, 13, 8, 3 and -4 cm from the mid of the 6 cm long sleeve sensor (Dentsleeve Pty Ltd, Belair, South Australia). The catheter was continuously perfused with gas free distilled water by a low compliance pneumohydraulic capillary infusion system (Arndorfer Medical Specialties, Greendale, Wisconsin, U.S.A.) at a rate of 0.5 ml/min. The external pressures transducers (Medex Inc., Ohio, U.S.A.) were connected via an analogue/digital converter (PC Polygraph HR, Synectics Medical, Stockholm, Sweden) to a personal computer system. The data were displayed continuously on a monitor and stored on the personal computer system (Polygram Upper GI 6.30, Gastrosoft Inc., Synectics Medical, Stockholm, Sweden).

The manometry catheter was introduced through the nose into the esophagus and positioned so that the sleeve sensor straddled the LES. The proximal side hole was positioned in the pharynx and was used for identification of swallow signals. The middle side holes registered esophageal body motility. The distal side hole was used as reference point for intragastric pressure. A glass pH electrode (Ingold LOT 440 continue glassreference electrode; Ingold Messtechnik AG, Urdorf, Germany) was passed through the nose and positioned 5 cm above the upper margin of the LES. The pH electrode had been calibrated at pH 4.0 and pH 7.0.

Study protocol

Each test was performed on a separate day in random order with an interval of at least seven days. The experiments were started at 08.30 h. after an overnight fast. The subjects were studied in the upright position, sitting in a comfortable chair. The manometry and pH catheter were introduced into the esophagus and positioned as described above. Two intravenous cannulas were inserted into the antecubital vein of each arm, one for intravenous

infusion, the other for blood sampling. L-arginine (L-arginine.HCL 10%) was given intravenously starting with a bolus of 0.625 ml/kg followed by continuous infusion of 2.1875 ml/kg.h for 120 min (total 500 mg/kg bodyweight). In the control experiments saline was administered intravenously.
At regular intervals (-60, 0, 30, 60, 120, 180 min) three wet swallows with 5 ml of water were given to determine swallow induced LES relaxations. Blood samples for determination of plasma hormone levels were taken from -60 min to 180 min at regular intervals (-60, -30, 0, 15, 30, 60, 90, 120, 180 min). Plasma cholecystokinin (CCK) and gastrin concentration were determined by radioimmunoassay [14,15]. Blood samples to determine plasma levels of amino acids arginine en citrulline were drown at regular intervals: fasting (0 min), after start of infusion (30 min), at the end of infusion (120 min) and one hour after end of infusion (180 min). Plasma levels of amino acids arginine en citrulline were measured as described previously [16].

Fasting protocol

Two tests were performed in random order under fasting conditions [control vs L-arginine (ARG)]. Esophageal pH and motility were registered simultaneously for one hour under basal, fasting conditions (time -60 to 0 min) followed by two hours (time 0 to 120 min) during infusion of L-arginine or saline (control). After the end of the infusion esophageal pH and motility were registered for one more hour (time 120 to 180 min).

Fed protocol

Two tests were performed in random order after ingestion of a meal (control-meal vs ARG-meal). The carbohydrate rich - high fat meal consisted of 200 g bananas blended with 125 ml cream and 25 ml Roosvicee (Koninklijke De Ruiter, Baarn, The Netherlands); (5 g protein, 44 g fat and 59 g carbohydrates, 2722 kJ). Esophageal pH and motility were registered simultaneously for one hour under basal, fasting conditions (time -60 to 0 min) followed by two hours (time 0 to 120 min) during infusion of L-arginine or saline (control). The meal was given 15 min after start of the infusion. Subjects were asked to consume the meal within 10 min. After the end of the infusion esophageal pH and motility were registered for one more hour (time 120 to 180 min).

Data analysis

Lower esophageal sphincter

Lower esophageal sphincter tracings were analyzed for LES resting pressure (LESP) and LES relaxations (LESR). LESP was defined as mean end-expiratory LESP relative above intragastric pressure over a 2 min period. LESR are divided in swallow induced LESR and spontaneous LESR. Swallow induced LESR are preceded by active swallows starting with a pharyngeal contraction. Residual LESP after wet swallows was defined as end-expiratory nadir LESP above intragastric pressure. Transient LES relaxation (TLESR) are defined as spontaneous decreases in LESP of $\geq$ 5 mmHg with a rate of $\geq$ 1 mmHg/sec, within 10 sec reaching a pressure of $\leq$ 2 mmHg above intragastric pressure. No swallow signal occurs in the interval from 4 sec before to 2 sec after onset of LESR. Swallow related LESR are defined TLESR, irrespective of the timing of LESR to swallowing when the duration of LESR is at least 10 sec [10].

pH analysis

Gastroesophageal reflux episodes are defined as a sudden fall of pH below 4.0 with a duration of at least 4 sec. The number and duration of reflux episodes were counted.
The mechanisms of each reflux episode were scored using the following criteria. Gastroesophageal reflux occurred during:
(1) TLESR (spontaneous LESR meeting the earlier mentioned criteria; swallow related LESR with the duration of LESR ≥ 10 sec)
(2) Swallow induced LESR (primary peristalsis or failed primary peristalsis with the duration of LESR ≤ 10 sec or multiple swallowing).
(3) LES pressure drift (a gradual loss of basal LES pressure).
(4) Absent LES pressure (LESP less than 2 mmHg above intragastric pressure).
(5) Abdominal strain (an increase in abdominal pressure).

Statistical analysis

Data are expressed as mean values ± SEM. Data were analyzed for statistical significance using multiple analysis of variance (MANOVA). When this indicated a probability of less than 0.05 for the null hypothesis, Student-Newman-Keuls analyses were performed to determine which values between or within the experiments differed significantly. A p value of <0.05 was considered significant for all analyses.

RESULTS

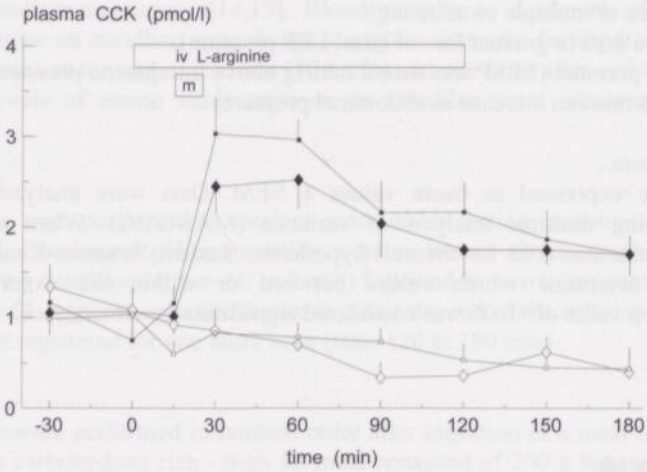
Plasma amino acids

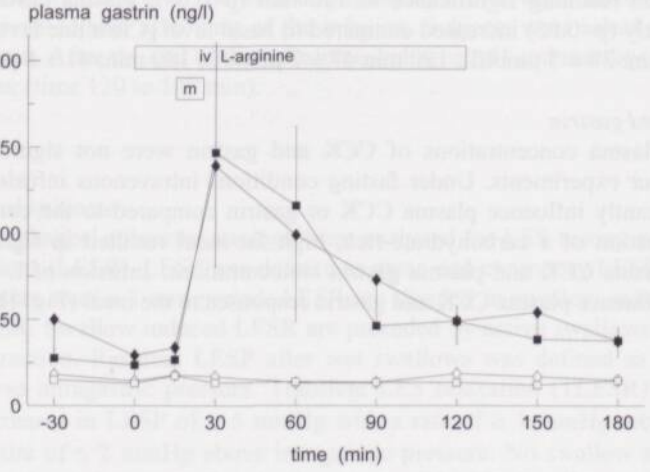
Intravenous infusion of L-arginine significantly ($p<0.01$) increased plasma arginine levels over basal (L-arginine levels at basal: 45 ± 9 μmol/L; 30 min: 1106 ± 132 μmol/L; 120 min 1758 ± 228 μmol/L; 180 min: 568 ± 108 μmol/L). Plasma citrulline increased during L-arginine infusion reaching significance at 120 min ($p<0.05$). Plasma citrulline increments were significantly ($p<0.05$) increased compared to basal level (Citrulline levels at basal: 31 ± 5 μmol/L; 30 min: 39 ± 5 μmol/L; 120 min 47 ± 7 μmol/L; 180 min: 41 ± 5 μmol/L).

Plasma CCK and gastrin

Basal plasma concentrations of CCK and gastrin were not significantly different between the four experiments. Under fasting conditions intravenous infusion of L-arginine did not significantly influence plasma CCK or gastrin compared to the control experiment (Fig. 1A). Ingestion of a carbohydrate-rich, high fat meal resulted in significant ($p<0.01$) increases in plasma CCK and plasma gastrin concentrations. Infusion of L-arginine did not significantly influence plasma CCK and gastrin responses to the meal (Fig. 1B).

Figure 1A-B. *Plasma levels (mean ± SEM) of [A] cholecystokinin (CCK) and [B] gastrin during control (open squares) or continuous infusion of L-arginine (open diamonds). L-arginine was given intravenously in a dosage of 500 mg/kg in 120 min. Six healthy volunteers were studies under fasting conditions. Six other healthy volunteers were studied after ingestion of a carbohydrate high fat meal (M) (closed squares and closed diamonds).*





Esophageal body motility

Esophageal body contractions and peristalsis were not significantly influenced by intravenous L-arginine neither under fasting conditions nor under postprandial conditions. For the distal esophageal body contractions, the amplitude was 69 ± 8 mmHg versus 67 ± 4 mmHg (arginine vs control), the duration was 3.2 ± 0.1 versus 3.3 ± 0.2 s. and the velocity was 4.7 ± 0.3 versus 4.0 ± 0.2 cm/s.

Swallow induced LES relaxation

Swallow induced LES relaxations were complete in all experiments. Residual LESP after 5 ml wet swallows was similar during ARG (≤ 1 mmHg) to that during control (≤ 1 mmHg). Residual LESP after ingestion of a meal was similar during ARG-meal (≤ 1 mmHg) and during control-meal (≤ 1 mmHg). The duration of LES relaxations during ARG and control were not significantly different (4.0 ± 0.3 sec vs 4.3 ± 0.2 sec). The duration of LES relaxation after ingestion of a meal was not influenced during ARG-meal (4.7 ± 0.4 sec) compared to control-meal (4.7 ± 0.3 sec).

Lower esophageal sphincter pressure

LESP in the basal period (-60 to 0 min) was not significantly different between the control and ARG experiment. During two hours of intravenous infusion of L-arginine there were no significant changes in LESP compared to the control experiment (Fig. 2A). LESP in the basal period was not significantly different between the control-meal and ARG-meal experiment. After ingestion of the fat-rich meal LESP decreased significantly ($p<0.01$) compared to basal level from t=20 to t=180 min. Intravenous infusion of l-arginine had no significant effect on LESP compared to the control-meal experiment (Fig. 2B).

Transient lower esophageal sphincter relaxation

The frequency of TLESR was not significantly different between the basal periods of the four experiments (Table 1). During the control experiment no significant changes in TLESR frequencies were observed. Intravenous infusion of L-arginine had no significant effect on the frequency of TLESR compared to the control experiment. After ingestion of the meal the frequency of TLESR significantly ($p<0.05$) increased in the first postprandial hour (4.5 ± 0.8 TLESR/h). During L-arginine infusion the postprandial increase of TLESR (5.3 ± 1.3 TLESR/h) was not significantly different compared to meal-control experiment. The duration of TLESRs was not significantly different between the four experiments (control: 15.3 ± 1.1 sec; ARG: 17.3 ± 1.9 sec; control-meal: 17.6 ± 1.7 sec; ARG-meal: 16.7 ± 0.3 sec).

Figure 2A-B. *Lower esophageal sphincter pressure (LESP) (mean ± SEM) during control (open squares) or continuous intravenous infusion of L-arginine (open diamonds) in six healthy volunteers under [A] fasting and [B] postprandial conditions. L-arginine was given intravenously in a dosage of 500 mg/kg in 120 min. The 400 ml carbohydrate high fat meal (M) consisted of 5 g protein, 44 g fat and 59 g carbohydrates (closed squares and closed diamonds).*

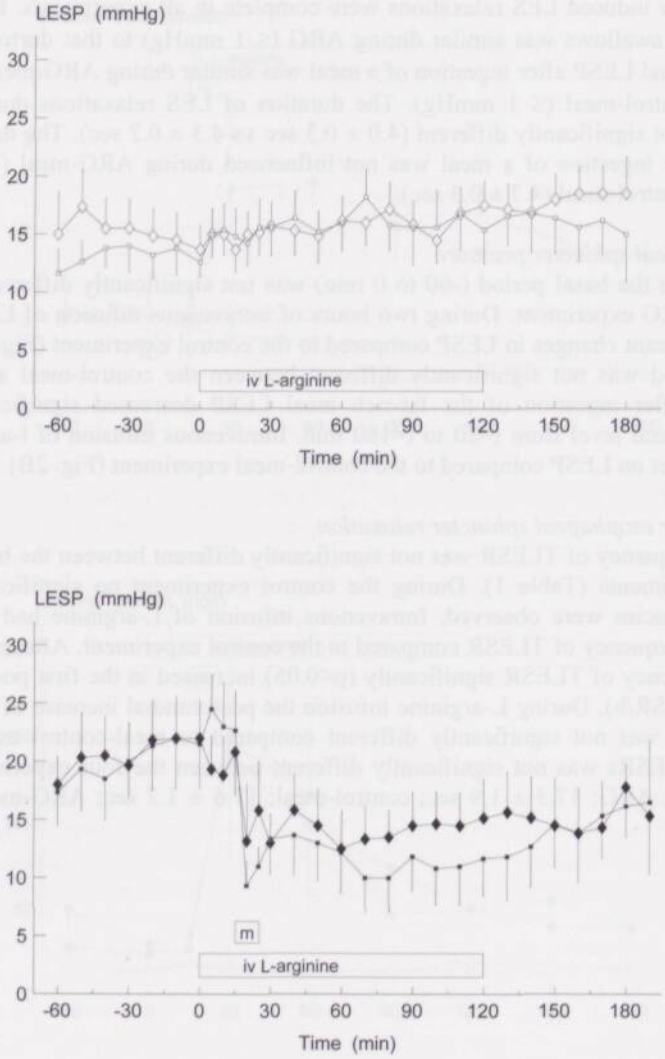


Table 1. *Frequency of TLESR (number/hour; mean±SEM) one hour before (basal) and during three hours of intravenous infusion of saline (control) or arginine (ARG) in six healthy subjects under fasting and postprandial conditions. Asterisks denote a significant (p<0.05) increase compared to basal.*

TLESR	control	ARG	control-meal	ARG-meal
Basal	3.0±0.8	1.5±0.6	2.7±0.7	2.8±1.1
Infusion				
I	1.5±0.4	2.7±0.6	4.5±0.8*	5.3±1.3*
II	2.7±0.5	2.7±0.6	3.5±0.7	3.0±1.2
III	2.7±0.3	2.8±0.5	2.8±1.0	3.2±1.0

Mechanisms of gastroesophageal reflux

As expected, the number of reflux episodes and the percentage of time pH<4 was low in the healthy volunteers even after meal ingestion. In all experiments the predominant mechanism of reflux were TLESRs (80%). (Table 2).

Table 2. *Mechanisms of gastroesophageal reflux during three hours of intravenous infusion of saline (control) or arginine (ARG) in six healthy subjects under fasting and postprandial conditions.*

Mechanisms of GER	control	ARG	control-meal	ARG-meal
% time pH <4	0.28%	0.58%	0.55%	0.57%
TLESR	11 (69%)	19(83%)	21(73%)	15(100%)
Swallow induced LESR	2(13%)	0(0%)	3(10%)	0(0%)
LES pressure drift	1(6%)	4(17%)	2(7%)	0
Absent LES pressure	0	0	1(3%)	0
Abdominal strain	1(6%)	0(0%)	1(3%)	0(0%)
Other	1(6%)	0(0%)	1(3%)	0
Total	16(100%)	23(100%)	29(100%)	15(100%)

DISCUSSION

Ingestion of a fat-rich meal significantly reduced LESP and significantly increased the postprandial frequency of TLESR. Intravenous infusion of L-arginine did not significantly influence LES pressure or TLESRs neither under fasting nor under postprandial conditions. Swallow induced relaxations of the LES and TLESRs were not affected by L-arginine.

The amount of L-arginine that can be administered intravenously without vascular effects is limited. The total dose of L-arginine we administered may affect blood pressure or heart rate when administered in a 30 min period [17]. We have administered the total dosage in 120 min to avoid systemic effects that might influence LES. L-arginine administration resulted in increases in the plasma levels of L-citrulline pointing to increased NO production.

It is currently not possible to constitute in vivo direct proof of stimulation of NO synthesis by L-arginine infusion in man, because of the short half-life of NO caused by oxidation to NO2- and NO_3^- [18].

The effect of L-arginine may very much depend on the study conditions of L-arginine may, under certain circumstances, increase agonist-stimulated NO synthesis. In animal experiments LES relaxation due to electrical nerve stimulation is antagonized by inhibitors of NO biosynthesis. The effect of NO synthase inhibitors is prevented by L-arginine. These studies provided evidence that NANC relaxations are mediated by NO. Similar experiments with human smooth muscle specimens taken from the esophagogastric junction showed consistent results that inhibitory NANC responses to electrical stimulation of nerves in human LES are mediated by a product of L-arginine/nitric oxide pathway.

Willis *et al* [19] have studied the effect of a NO donor (Molsidomin) on LES. The NO donor decreased basal LESP and slightly reduced amplitude and peristaltic velocity of dry swallows. However LES responses to wet swallows were not affected. These results are consistent with our findings that infusion of the precursor of NO, L-arginine did influence esophageal motility after wet swallows. Luiking *et al* [20] have studied the effect of long-term oral L-arginine supplementation on LES motility. A daily dose of 30 g for 8 days suppressed the late postprandial LESP increase. The frequency of TLESR was not affected by L-arginine but the mean duration of TLESR was prolonged. Plasma levels of L-arginine after oral intake of L-arginine were much lower compared to intravenous infusion. In the present study, even during acute increases of plasma L-arginine we did not find significant changes in TLESR characteristics. TLESRs are spontaneous LES relaxations not related to swallowing. The exact mechanism of stimulation of TLESR is not known. Fat containing meals provoke TLESR. This effect may be mediated via CCK while ingestion of fat increases plasma CCK levels. The putative role of CCK on TLESR is under investigation. CCK-33 significantly reduced LES pressure but the postprandial frequency of TLESR was not influenced by intravenous CCK-33 [21]. CCK-8 increased the frequency of TLESR during gastric distension with an intragastric barostat bag [22] whereas a CCK-A receptor antagonist inhibited the frequency of TLESR [22] Boulant *et al* reported that triggering of TLESR by gastric distension is inhibited not only by CCK-A receptor antagonist but also by a NO synthase inhibitor under stimulatory conditions [13]. It was than suggested that CCK may be involved in the occurrence of TLESR through peripheral CCK-A receptors and an L-arginine nitric oxide pathway. This study shows that, after stimulation of endogenous CCK, addition of the precursor of NO, L-arginine, did not increase the frequency of TLESR.

In conclusion, we have studied the effect of intravenous infusion of L-arginine on LES characteristics. Neither under fasting nor under postprandial conditions did L-arginine significantly influence LESP, TLESRs or esophageal motility.

REFERENCES

1. Sanders KM, Ward SM. Nitric oxide as a mediator of nonadrenergic noncholinergic neurotransmission. Am J Physiol 1992; 262:G379-G392.
2. Stark ME, Szurszewski JH. Role of nitric oxide in gastrointestinal and hepatic function and disease. Gastroenterology 1992; 103:1928-1949.
3. Richards WG, Sugarbaker DJ. Neuronal control of esophageal function. Chest Surg Clin N Am 1995; 5:157-171.
4. Bult H, Boeckxstaens GE, Pelckmans PA, Jordaens FH, Van Maercke YM, Herman AG. Nitric oxide as an inhibitory non-adrenergic non-cholinergic neurotransmitter. Nature 1990; 345:346-347.
5. De Man JG, Pelckmans PA, Boeckxstaens GE, et al. The role of nitric oxide in inhibitory non-adrenergic non- cholinergic neurotransmission in the canine lower oesophageal sphincter. Br J Pharmacol 1991; 103:1092-1096.
6. Anand N, Paterson WG. Role of nitric oxide in esophageal peristalsis. Am J Physiol 1994; 266:G123-G131.
7. Yamato S, Saha JK, Goyal RK. Role of nitric oxide in lower esophageal sphincter relaxation to swallowing. Life Sci 1992; 50:1263-1272.
8. Preiksaitis HG, Tremblay L, Diamant NE. Nitric oxide mediates inhibitory nerve effects in human esophagus and lower esophageal sphincter. Dig Dis Sci 1994; 39:770-775.
9. Tottrup A, Ny L, Alm P, Larsson B, Forman A, Andersson KE. The role of the L-arginine/nitric oxide pathway for relaxation of the human lower oesophageal sphincter. Acta Physiol Scand 1993; 149:451-459.
10. Mittal RK, Holloway RH, Penagini R, Blackshaw LA, Dent J. Transient lower esophageal sphincter relaxation. Gastroenterology 1995; 109:601-610.
11. Dodds WJ, Dent J, Hogan WJ, et al. Mechanisms of gastroesophageal reflux in patients with reflux esophagitis. N Engl J Med 1982; 307:1547-1552.
12. Holloway RH, Kocyan P, Dent J. Provocation of transient lower esophageal sphincter relaxations by meals in pasophageal sphincter relaxations by meals in patients with symptomatic gastroesophageal reflux. Dig Dis Sci 1991; 36:1034-1039.
13. Boulant, J., Fioramonti, J., Dapoigny, M., Bommelaer, G.,Bueno, L. Cholecystokinin and nitric oxide in transient lower esophageal sphincter relaxation to gastric distention in dogs. Gastroenterology 1994; 107:1059-1066.
14. Jansen, J.B.,Lamers, C.B.H.W. Radioimmunoassay of cholecystokinin in human tissue and plasma. Clin Chim Acta 1983; 131:305-316.
15. Jansen, J.B.,Lamers, C.B.H.W. Effect of changes in serum calcium on secretin-stimulated serum gastrin in patients with Zollinger-Ellison syndrome. Gastroenterology 1982; 83:173-178.
16. Pijl, H., Koppeschaar, H.P., Cohen, A.F., Iestra, J.A., Schoemaker, H.C., Frolich, M., Onkenhout, W.,Meinders, A.E. Evidence for brain serotonin-mediated control of carbohydrate consumption in normal weight and obese humans. Int J Obes Relat Metab Disord 1993; 17:513-520.
17. Smulders, R.A., Stehouwer, C.D., Olthof, C.G., Van Kamp, G.J., Teerlink, T., De Vries, P.M.,Donker, A.J. Plasma endothelin levels and vascular effects of intravenous L- arginine infusion in subjects with uncomplicated insulin- dependent diabetes mellitus. Clin Sci 1994; 87:37-43.
18. Bode-Boger, S.M., Boger, R.H., Creutzig, A., Tsikas, D., Gutzki, F.M., Alexander, K.,Frolich, J.C. L-arginine infusion decreases peripheral arterial resistance and inhibits platelet aggregation in healthy subjects. Clin Sci 1994; 87:303-310.

19. Willis, S., Allescher, H.D., Stoschus, B., Schusdziarra, V., Classen, M.,Schumpelick, V. Double blind placebo controlled study on the effect of the nitric oxide donor molsidomin and the 5-HT3 antagonist ondansetron on human esophageal motility. Z Gastroenterol 1994; 32:632-636.
20. Luiking, Y.C., Weusten, B.L., Portincasa, P., Van Der Meer, R., Smout, A.J.,Akkermans, L.M. Effects of long-term oral L-arginine on esophageal motility and gallbladder dynamics in healthy humans. Am J Physiol 1998; 274:G984-G991.
21. Ledeboer, M., Masclee, A.A.M., Batstra, M.R., Jansen, J.B.,Lamers, C.B.H.W. Effect of cholecystokinin on lower oesophageal sphincter pressure and transient lower oesophageal sphincter relaxations in humans. Gut 1995; 36:39-44.
22. Boulant J, Mathieu S, D'Amato M, Abergel A, Dapoigny M, Bommelaer G. Cholecystokinin in transient lower oesophageal sphincter relaxation due to gastric distension in humans. Gut 1997; 40:575-581.

9

COMPARISON OF TWO TECHNIQUES FOR LOWER ESOPHAGEAL SPHINCTER MANOMETRY: SLEEVE AND SPHINCTOMETER

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Neurogastroenterol Motil 2004; 16:265-268

ABSTRACT

Background and methods: We have compared the sphinctometer with the water-perfused sleeve (gold standard) for measurement of lower esophageal sphincter (LES) characteristics by simultaneous recording.

Results: LES pressure and transient LES relaxations (TLESR) measured by sleeve and sphinctometer in eleven healthy volunteers showed identical patterns. However, output of the sphinctometer was significantly ($p<0.01$) lower than output of the sleeve. A total of 249 TLESR were recorded.. Of these, 176 TLESR were identified by both sleeve and sphinctometer, 50 TLESR were identified by sleeve alone and 23 TLESR by sphinctometer alone. Due to the lower pressure output of the sphinctometer, 29 LES relaxations did not reach amplitude criteria to qualify as TLESRs. When TLESR criteria were adjusted for sphinctometer pressure measurements, the number of TLESRs identified by both sleeve and sphinctometer increased from 176 to 205.

Conclusions: In healthy volunteers the sphinctometer registers TLESR with results comparable to sleeve recording. However at low LES pressures, the number of TLESR is underscored by the sphinctometer.

INTRODUCTION

In patients with reflux disease and in healthy subjects transient lower esophageal sphincter relaxations (TLESR) are the major reflux mechanism(1). The water-perfused sleeve designed by Dent (2) is used to record TLESR during stationary or ambulatory studies. Gotley et al. described the sphinctometer, an oil-filled cylinder containing a pressure microtransducer incorporated into an ambulatory esophageal solid state catheter. In vitro studies have shown that the sphinctometer adequately records LES pressure (3,4) However, when the length of the sphinctometer is only partially exposed to pressure, the absolute pressure recorded by the sphinctometer is lower than the applied pressure. In vivo, catheters incorporating a sphinctometer have been used for ambulatory LES pressure recording (5). However, until recently ambulatory recording of TLESR by the sphinctometer has not been performed. In one study with the sphinctometer, LES characteristics during acid reflux events were consistent with the concept of TLESR (4).

The aim of our study was to compare water-perfused sleeve manometry and solid state sphinctometer manometry for recording TLESRs during simultaneous measurements.

METHODS

Eleven healthy subjects (age 19-24 years; 6 females) without a history of gastro-intestinal disease or surgery participated in the study. Informed consent was obtained and the study had been approved by the local Ethics Committee.

Manometric and pH technique

Water-perfusion manometry (sleeve) was performed as described previously (6), using a multilumen silicone rubber tube (outer diameter 5.0mm) with 7 side-holes at 29, 23,

18, 13, 8, 3 and –4 cm from the mid-point of the 6 cm long sleeve sensor (Dentsleeve Pty Ltd, Belair, South Australia). Solid-state manometry (sphinctometer) was performed with a electronic catheter (Sentron, Medtronics, Roden, the Netherlands) with five solid-state pressure microtransducers at 30, 18, 8, 0 and –4 cm from the mid-point of the sphinctometer. The sphinctometer is an oil-filled silicone rubber cylinder around a solid-state pressure microtransducer, 6cm long and a diameter of 0.4 cm. The sphinctometer had been calibrated at 0 mmHg and at 50 mmHg. Data of sleeve catheter and sphinctometer were displayed continuously on a monitor and stored on a personal computer system (Polygram Upper GI 6.30, Gastrosoft Inc., Medtronics, Denmark).

In vitro studies were performed in triplicate to test the sphinctometer susceptibility to pressure reading drifts during 24 hour recordings. The sphinctometer was calibrated at 20 °C and thereafter submerged into a warm water bath of 37 °C for recording. Changes in pressure reading of the sphinctometer and microtransducers were recorded on a portable recorder (μdigitrapper, Medtronics, Denmark) and processed afterwards. In vitro studies with the sphinctometer showed that during 24 hours at a constant temperature of 37oC the drift in output of the sphinctometer was <1 mmHg.

Study protocol

Experiments were started at 8.30 a.m. with subjects fasted overnight. The sleeve catheter was positioned so that the sleeve sensor straddled the LES. Side holes were used for recording pharyngeal swallow signals, esophageal body motility, and intragastric pressure. The sphinctometer was placed at exactly the same position as the sleeve. A glass pH electrode (Ingold LOT 440; Ingold Messtechnik AG, Urdorf, Germany; calibrated at pH 4.0 and pH 7.0) was positioned 5 cm above the upper margin of the LES. Esophageal pH and motility were recorded simultaneously for one hour under fasting conditions (time -60 to 0min) and for three hours (0 to 180min) after ingestion of a 400 ml liquid meal (Nutridrink; Numico, Zoetermeer, The Netherlands) containing 20g protein, 26g fat and 72g carbohydrates (2520 kJ).

Data analysis

Lower esophageal sphincter tracings were analyzed for LES resting pressure and TLESR. The TLESR were defined according to Holloway et al.(1,7) Gastro-esophageal reflux episodes and reflux mechanisms were scored using criteria described previously(6). Data are expressed as mean ± SEM. The Wilcoxon signed ranks test was used to compare results between sleeve and sphinctometer. Data were analyzed using MANOVA. When this indicated a probability of less than 0.05 for the null hypothesis, Student-Newman-Keuls analyses were performed to determine which values between or within the experiments differed significantly ($p<0.05$).

RESULTS

Lower esophageal sphincter pressure

LES pressure and LES relaxations measured by sleeve and sphinctometer showed an identical pattern (Figure 1, tracing A). However, the output of the sphinctometer was significantly ($p<0.01$) lower than the output of the sleeve (Figure 2)from 15 to 180 minutes after meal ingestion LES pressure decreased significantly ($p<0.01$) compared to basal level, and this was identified with both recording techniques.

Transient lower esophageal sphincter relaxations

In total 249 TLESR were identified by the sleeve and/or the sphinctometer. Of these, 176 TLESR (71%) were identified by both sleeve and sphinctometer, 50 by sleeve alone (20%) TLESR, and 23 by sphinctometer alone (9%) TLESR. Recordings were analyzed in detail (Table 1). Twenty-nine LES relaxations not detected by the sphinctometer had the typical characteristics of a TLESR except for one or two criteria: relaxation rate and absolute fall in pressure did not fulfill the strict criteria of TLESR described by Holloway *et al* (7) especially when LES pressure was low (<5 mmHg). Adjustment of the criteria for TLESR improved identification of TLESR by the sphinctometer. Thus, with minimum LESP decrease set at ≥4 mmHg and relaxation rate at ≥0.4 mmHg/s , 195 TLESRs were scored by both sleeve and sphinctometer. Four episodes were identified by sphinctometer as 'drift of LES pressure' instead of TLESR because the rate of LES pressure decrease was less than 1.0 mmHg/sec (recorded value 0.4 mmHg/sec.) With minimum LES pressure decrease set at ≥3 mmHg and relaxation rate of ≥0.3 mmHg/s, 205 TLESRs were scored by both sleeve and sphinctometer. With other cut-offs, the number of identified TLESR was not higher, and visual identification became more difficult.

The duration of TLESR measured by the two different catheters correlated significantly (r_s: $p<0.001$) and was slightly longer as measured by sleeve (16.5±0.4s) compared to sphinctometer (14.9±0.4s,$p<0.05$).

Table 1. *The tracings at time of a TLESR, identified by either sleeve or sphinctometer, were analyzed in detail to determine the criteria on which proper qualification had failed by sleeve or sphinctometer. The main characteristics of each LES relaxation that was not identified as TLESR by sleeve or sphinctometer are described (results in numbers).*

	Sleeve	Sphinctometer
failed to quality as TLESR	23	50
Relaxation rate <1 mmHg/s	3	22
LESP decrease <5 mmHg	0	7
LES nadir pressure >2 mmHg	10	12
Swallow related LESR	8	9
Refill water container	2	0

Figure 1. *Transient lower esophageal sphincter relaxations (arrows) during simultaneous measurement by water-perfused sleeve and sphinctometer in healthy subjects. The tracings consist of esophageal pH (1), pharyngeal pressure (2), esophageal body pressure (3,4), LES pressure (5-sleeve, 6-sphinctometer) and intragastric pressure (7).*
Tracing A: Both tracings reveal identical patterns, however, the output of the sphinctometer is significantly lower than the output of the sleeve sensor.
Tracing B: Although the tracings reveal identical patterns, the output of the sphinctometer does not fulfill the criteria of a TLESR because the rate of relaxation is less than 1.0 mmHg/s. This TLESR is associated with acid reflux.
Tracing C: After a peristaltic wave in response to multiple wet swallows (S) a prolonged LES relaxation is registered by the sphinctometer. The sphinctometer output is scored as a TLESR, but the tracing of the sleeve does not fulfill the criteria of TLESR. The nadir pressure is >2 mmHg above intragastric pressure.

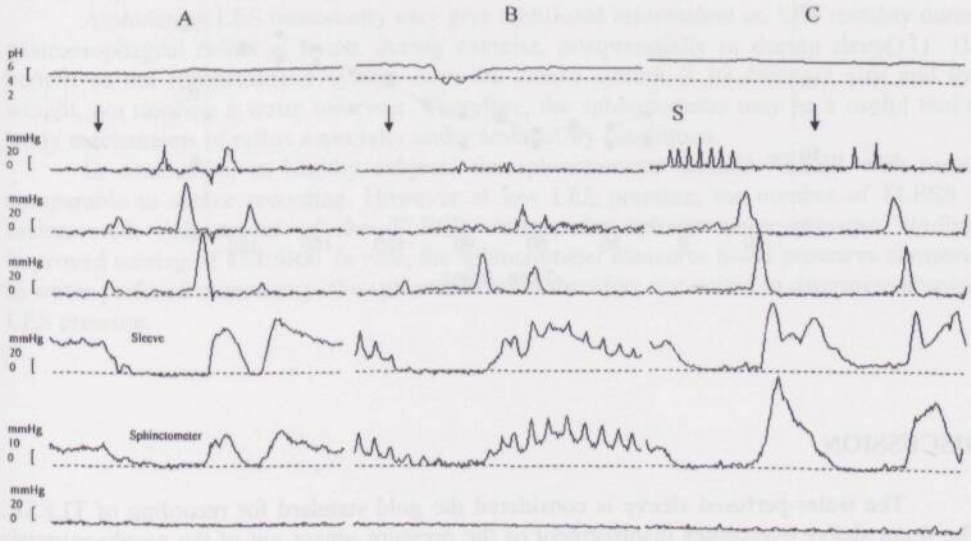
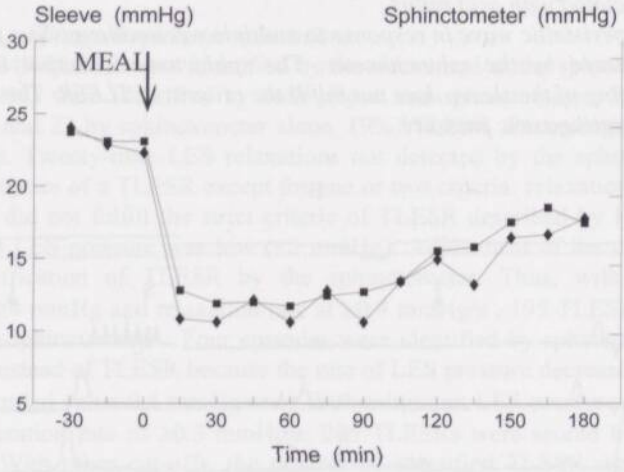


Figure 2. *Lower esophageal sphincter pressure (mean ± SEM) in eleven healthy volunteers under fasting conditions and after ingestion of a meal (2520 kJ) at t=0 min.*
The output of the sphinctometer (diamonds) is significantly lower than the output of the sleeve (squares). The output of sleeve and sphinctometer are plotted on different scales to show an identical postprandial decrease.



DISCUSSION

The water-perfused sleeve is considered the gold standard for recording of TLESR. The 6 cm sleeve overcomes displacement of the pressure sensor out of the esophagogastric junction during respiration, peristalsis etc. The catheter incorporating the sphinctometer was constructed according to the same specifications as the water-perfused sleeve catheter: a 6 cm long sensor, with a pressure sensor for swallow signals and an intragastric pressure sensor necessary for identification of TLESR (1). LES pressures recorded with the sphinctometer were lower than those with the sleeve. This difference did not result from the position of the catheter in the LES. A catheter in the left lateral position measures higher pressures because of the radial asymmetry of the LES (8) In our study positioning of the catheters occurred randomly. The differences in LES pressure appear more likely to be related to the techniques themselves. The sleeve records the highest external pressure at any point along the sensor (2,9), whereas the sphinctometer accurately records pressure only when the whole surface of the sphinctometer is exposed to an external pressure (3,4). Partial exposure of the sphinctometer reduces the output depending on the length of the sphinctometer exposed in a linear way. The sphinctometer is calibrated during total (6 cm) surface exposure in a water

column. However, the length of the LES segment covering the sphinctometer in vivo is usually less than 6 cm. It therefore appears inherent that the output of this sphinctometer will be lower than the exposed pressure.

LES relaxations were recognized by both sleeve and sphinctometer. Using the sleeve criteria (7) for analysis of the sphinctometer recording, 50 spontaneous LES relaxations were not classified as TLESR, and this was most apparent in subjects with low postprandial spontaneous LES relaxations. Adjustment of two of the criteria postulated by Holloway *et al* (7) markedly improved TLESR identification by the sphinctometer: 1) abrupt falls of LES pressure within 10 sec reaching a pressure of ≤3 mmHg above intragastric pressure and 2) a relaxation rate ≥0.3 mmHg/s. With these criteria, over 90% of TLESR measured by sleeve were also classified as TLESR by the sphinctometer. Trudgill *et al* (10) compared sleeve and sphinctometer during simultaneous manometry. Based upon a small number of TLESR analyzed (N=28), they also concluded that the sphinctometer has a low capacity to register TLESR especially at low LES pressures.

Ambulatory LES manometry may give additional information on LES motility during gastroesophageal reflux at home, during exercise, postprandially or during sleep(11). The benefit of the sphinctometer system over the sleeve system is its compact size and low weight, not needing a water reservoir. Therefore, the sphinctometer may be a useful tool to study mechanisms of reflux especially under ambulatory conditions.

In conclusion, in healthy subjects the sphinctometer records TLESR with results comparable to sleeve recording. However at low LES pressure, the number of TLESR is underscored. Adjustment of the TLESR criteria for sphinctometer pressure readings improved scoring of TLESRs. *In vivo*, the sphinctometer measures lower pressures compared to water-perfused manometry, the sphinctometer is therefore not suited to determine absolute LES pressure.

REFERENCES

1. Mittal RK, Holloway RH, Penagini R, Blackshaw LA, Dent J. Transient lower esophageal sphincter relaxation. Gastroenterology 1995; 109: 601-610.
2. Dent J. A new technique for continuous sphincter pressure measurement. Gastroenterology 1976; 71: 263-267.
3. Gotley DC, Barham CP, MillerR, Arnold R, Alderson D. The sphinctometer: a new device for measurement of lower oesophageal sphincter function. Br J Surg 1991; 78: 933-935.
4. Pehlivanov N, Liu J, Arora T, Yamamoto Y, Mittal RK. Lower esophageal sphincter monitoring with sphinctometer: in vitro and in vivo studies. Am J Physiol 1999; 277: G577-G584.
5. Barham CP, Gotley DC, Mills A, Alderson D. Precipitating causes of acid reflux episodes in ambulant patients with gastro-oesophageal reflux disease. Gut 1995; 36: 505-510.
6. Masclee AAM, Hoback JMLM, Ledeboer M, Lamers CBHW, Goozen HG. Prospective study of the effect of the Belsey Mark-IV fundoplication on reflux mechanism. Scan J Gastroenterol 1998; 33:905-910.
7. Holloway RH, Penagini R, Ireland AC. Criteria for objective definition of transient lower esophageal sphincter relaxation. Am J Physiol 1995; 268: G128-G133.
8. Schneider JH, Crookes PF, Becker HD. Four-channel sleeve catheter for prolonged measurement of lower esophageal sphincter pressure. Dig Dis Sci 1999; 44: 2456-2461.
9. Linehan JH, Dent J, Dodds WJ, Hogan WJ. Sleeve device functions as a Starling resistor to record sphincter pressure. Am J Physiol 1985; 248: G251-G255.
10. Trudgill NJ, Riley SA. Monitoring the lower esophageal sphincter – sphinctometer or sleeve. Neurogastroenterol Motil 1999; 11: 173-174.
11. Schoeman MN, Tippett MD, Akkermans LM, Dent J, Holloway RH. Mechanisms of gastroesophageal reflux in ambulant healthy human subjects. Gastroenterology 1995; 108: 83-91.

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SPHINCTOMETER RECORDING OF LES PRESSURE AND TRANSIENT LES RELAXATIONS IN PATIENTS WITH REFLUX DISEASE

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Acceptence for publication Neurogastroenterol Motil

ABSTRACT

Background: Investigate LES characteristics such as LES pressure and transient lower esophageal sphincter relaxations (TLESRs) in patients with gastroesophageal reflux disease (GERD) under physiological, ambulatory conditions.
Methods: Ambulatory 24 hr esophageal pH- and manometry by sphinctometer was performed in 15 GERD patients (5 females, age 26 - 75 yrs) and 10 healthy controls (5 females, age 19 - 55 yrs). Meal ingestion and supine period (sleep) were standardized.
Results: A circadian pattern was found for LES pressure with higher LES pressure supine (night) vs upright (day). At night but not at daytime, LES pressure was significantly ($p<0.01$) lower in patients vs controls. In patients the TLESR frequency was significantly ($p<0.01$) lower compared to controls, upright (3.2 ± 0.2 vs 2.2 ± 0.1 TLESR/h) but not supine (both 1.3 ± 0.1 TLESR/h). The percentage of TLESRs associated with reflux was significantly higher in GERD patients versus controls ($49 \pm 8\%$ vs $28 \pm 7\%$; $p<0.05$). TLESRs were the major reflux mechanism both in GERD patients (51%) and controls (71%).
Conclusions: A circadian pattern is observed for LES pressure and TLESR frequency. When upright, TLESR frequency in GERD patients is significantly lower versus controls. In GERD patients significantly more TLESRs are associated with reflux.

INTRODUCTION

In patients with gastroesophageal reflux disease (GERD), acid reflux mainly occurs during transient lower esophageal sphincter relaxations (TLESRs) or when lower esophageal sphincter (LES) pressure is low (1-3). TLESRs as reflux mechanisms have been studied previously during standardized protocols but only for a limited period of several hours. Studies on mechanisms of gastroesophageal reflux that employ ambulatory instead of stationary conditions may better reflect normal daily routine and physiology. Results from investigations using an ambulatory water-perfused sleeve have confirmed that in healthy volunteers and patients with GERD, TLESRs indeed are the major mechanism of reflux (4,5). However, other mechanisms such as a hiatus hernia, a low LES pressure or swallow induced LES relaxations may contribute to the excess reflux (6). It has previously been suggested that patients with GERD have an increased frequency of TLESRs compared to healthy subjects (2,7-9). However, other investigators reported that the frequency of TLESRs is not significantly different between patients with GERD and controls (4,5,10-13).

The sphinctometer is a solid-state catheter especially designed for recording sphincter characteristics. It contains an oil-filled cylinder where a pressure transducer is incorporated. In vitro studies have shown that the pressure output of the sphinctometer when recording sphincter pressure is lower compared to water-perfused sleeve manometry (14-16). Simultaneous recording with sphinctometer and water-perfused sleeve revealed that the sphinctometer reliably and accurately records spontaneous LES relaxations (15-17). However, when basal LES pressure is low as is frequently the case in GERD patients, adjustment of the criteria for TLESR is required in order to classify these spontaneous LES relaxations as TLESRs (16). Aim of the present study was to obtain information on diurnal

variation in TLESRs and other reflux mechanisms by performing ambulatory 24 h sphinctometer recording. Both patients with GERD and healthy volunteers were studied.

METHODS

Subjects

Fifteen patients with GERD (5 females; age range 26 - 75 years) and ten healthy subjects (5 females; age range 19 - 55 years) participated in the study. GERD was proven by endoscopy (esophagitis) and/or by 24 hour ambulatory pH-metry. Severity of esophagitis was graded endoscopically according to criteria of Savary and Miller. Endoscopy showed esophagitis grade 0 in 4 patients, grade I in 2 patients, grade II in 6 patients. A hiatal hernia was found in 4 patients. Reflux medication was stopped at least 5 days prior to the test. None of the healthy subjects had a history of gastro-intestinal disease or surgery or was on chronic medication. Informed consent was obtained from each individual. The study had been approved by the Ethics Committee of the Leiden University Medical Center.

Manometric and pH technique

In all subject esophageal water-perfused sleeve manometry was performed to determine the position of upper and lower esophageal sphincter. Solid-state ambulatory manometry (sphinctometer) was performed with an electronic catheter containing a 6 cm long sphinctometer and five solid state pressure transducers located at 30, 18, 8, 0 and –4 cm from the midpoint of the sphinctometer. These transducers were used for identification of swallow signals, esophageal body motility and intragastric pressure (11). The sphinctometer is an oil-filled silicone rubber cylinder around a solid state pressure microtransducer, 6 cm long with a diameter of 0.4 cm. The sphinctometer had been calibrated at pressures of 0 and 50 mmHg.

The sphinctometer catheter was introduced through the nose into the esophagus and positioned so that the sphinctometer straddled the LES. A glass pH electrode (Ingold LOT 440 continue glass reference electrode; Ingold Messtechnik AG, Urdorf, Germany) was positioned 5 cm above the upper margin of the LES. The pH electrode had been calibrated at pH 4.0 and 7.0. Sphinctometer and pH-catheter were connected to an ambulatory recorder (µdigitrapper Medtronic, Denmark) for continuous data recording. At the end of the test data were transferred to a personal computer system for later analysis.

Study protocol

The recordings were started at 12.30-13.00 p.m. Esophageal pH and motility were recorded simultaneously during 24 consecutive hours. After 15-30 min under basal, fasting conditions esophageal motility was measured for three hours after ingestion of the meal (time 0 to 180 min) with subjects sitting in a comfortable chair. Meals were ingested after the start of the test around 13.00 h, around 18.00 h and around 9.00 h the following day. The standardized liquid lunch consisted of 400 ml Nutridrink (Nutricia, Zoetermeer, The Netherlands) and contained 20 g protein, 26 g fat and 72 g carbohydrates (2520 kJ). Subjects were asked to consume the meal within 10 min. Breakfast consisted of bread with cheese or marmalade and dinner consisted of meat, vegetables and boiled potatoes. Intake of food and drinks with pH below 4 was restricted. Studies were undertaken under ambulatory conditions on outpatient basis and each person was encouraged to follow the daily routine during

registration. At home subjects were in supine position from 23.00 - 07.00 hour. The next day subjects returned to the hospital at the end of the registration period. During the study, periods of eating and drinking and supine position were noted in a diary.

Data analysis

Data was divided in two upright periods (13.00-23.00 hour and 07.00-13.00 hour) and a supine period (23.00-07.00 hour). LES tracings were analyzed for LES pressure and TLESR. The TLESR were defined according to Holloway et al (18). Based on a previous report from our group, the following adjustments in TLESR scoring were made. During low (< 5 mmHg) sphinctometer output registration of LES pressure, LESR with all characteristics of TLESR but not fulfilling the above criteria concerning rate of relaxation or absolute fall in LES pressure were also scored as TLESR when 1) an abrupt fall of LES pressure within 10 sec reached a pressure of ≤ 3 mmHg above intragastric pressure and 2) a relaxation rate ≥0.3 mmHg/s with respect to the all other criteria for TLESR (16). Gastroesophageal reflux episodes and mechanisms of reflux were scored using criteria described previously (3,18).

Statistical analysis

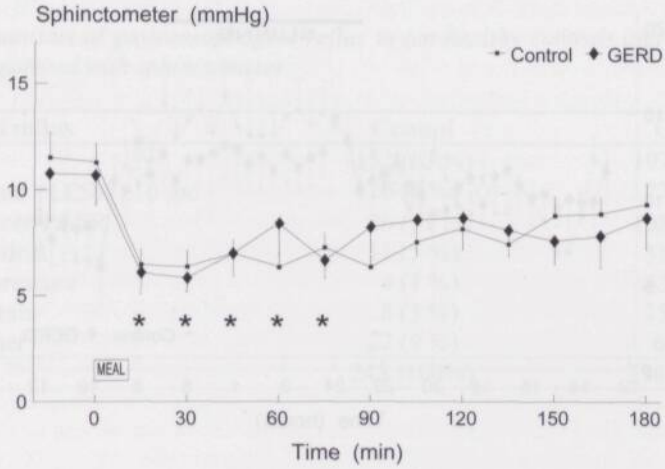
Data are expressed as mean ± SEM. LES pressure data were analyzed for statistical significance using (multiple) analysis of variance. The Mann Whitney U test for comparison of non-parametric data was used to compare results between patients and controls. A p value of <0.05 was considered significant.

RESULTS

Lower esophageal sphincter pressure

Fasting LES pressure (at 09.00 AM) was 12 ± 1 mmHg in controls and 11 ± 2 mmHg in patients with GERD. The meal significantly ($p<0.05$) decreased LES pressure in patients with GERD and controls up to 75 min after meal ingestion (Figure 1). LES pressure profiles during 24-hour sphinctometer registration showed LES pressure increments in the evening (Figure 2). In the control subjects LES pressure was significantly ($p<0.05$) higher during the supine period (14 ± 1 mmHg) compared to the upright period (10 ± 1 mmHg). In GERD patients LES pressure in the supine period (13 ± 2 mmHg) was higher compared to the upright period (11 ± 1 mmHg) although not significantly. In the supine period, but not in the upright period LES pressure was significantly ($p<0.01$) lower in GERD patients vs controls. In the second upright period, LES pressure again decreased to levels in the range of the previous day.

Figure 1. *Fasting and postprandial LES pressure in 15 patients with GERD (rhombs) and 10 healthy subjects (squares) *p< 0.05 postprandial versus fasting.*



Transient lower esophageal sphincter relaxation

TLESR frequency profile during 24 hour sphinctometer registration is shown in Figure 3. Fasting TLESR frequency (at 09.00 AM) was 2.5 ± 0.8 TLESR/h in controls and 1.8 ± 0.5 TLESR/h in patients with GERD. Meal ingestion significantly ($p<0.05$) increased the frequency of TLESR (controls: 4.8 ± 0.9 and GERD: 2.9 ± 0.5 TLESR/h). The frequency of TLESR in the upright period was significantly ($p<0.05$) lower in patients with GERD (2.2 ± 0.1 TLESR/h) compared to controls (3.2 ± 0.2 TLESR/h). During the supine period the frequency of TLESR was not significantly different between GERD patients and controls: 1.3 ± 0.2 vs. 1.3 ± 0.1 TLESR/h respectively. Both in patients and controls the number of TLESRs was significantly ($p<0.05$) lower supine vs. upright. The frequency of TLESRs was not significantly different between the first and second upright period (Table 1). The percentage of TLESRs associated with acid reflux was significantly ($p<0.05$) higher in patients with GERD compared to in controls (49 ± 8% vs. 28 ± 7%).

Figure 2. *Twenty four hour continuous registration of LES pressure in 15 patients with GERD (rhombs) and 20 healthy subjects (squares).*

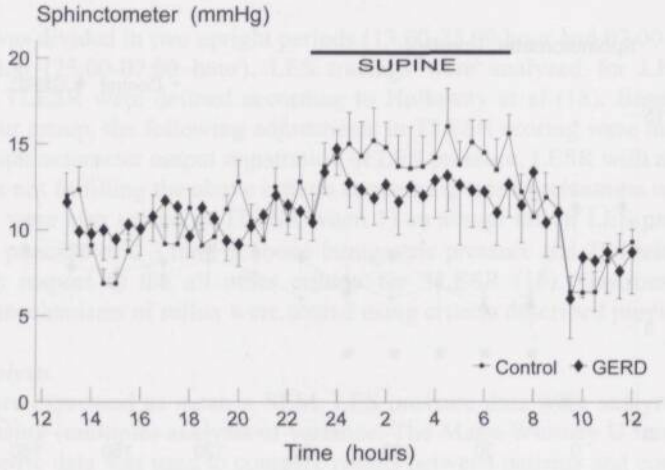


Table 1. *Diurnal frequency of TLESR (N/h) with comparison of upright periods and supine period in patients with GERD and controls. Asterisks denote significant (p<0.05) differences in the supine versus upright period. Dots denote significant (p<0.05) differences compared to controls.*

TLESR (N/h)	Control	GERD
13-23 hour	3.2 ± 0.2	2.2 ± 0.2•
23-07 hour	1.3 ± 0.2 *	1.3 ± 0.1*
07-13 hour	3.5 ± 0.2	1.9 ± 0.3•

Gastroesophageal reflux

The percentage of time with pH < 4 during 24 hr recording was significantly (p<0.05) higher in patients with GERD (7.0 ± 2.1 %) compared to controls (3.3 ± 0.9 %). This resulted both from a significantly higher number of reflux episodes in GERD patients vs controls (37 ± 5 vs 25 ± 5 per 24 hr) and from a significantly (p<0.05) longer duration of reflux episodes in patients with GERD versus controls (136 ± 12 sec vs 109 ± 11 sec).

Mechanisms of gastroesophageal reflux

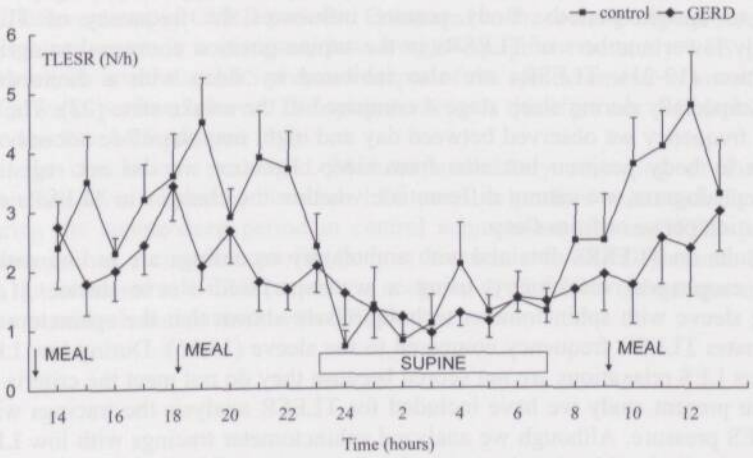
The mechanisms of reflux in patients and controls are listed in Table 2. TLESRs and swallow-related TLESRs are the main mechanism of reflux in control subjects (71%) and patients with GERD (51%). In patients with GERD about half of the reflux episodes occurred

during mechanisms of reflux other than TLESRs. Acid reflux episodes during swallow induced LES relaxation (20% vs. 11%), absent LES pressure (8% vs. 1%) and abdominal strain (14% vs. 3%) were increased compared to the control group. Twenty-eight episodes of acid reflux could not be explained by any of the mechanisms of reflux described above.

Table 2. *Mechanisms of gastroesophageal reflux in ten healthy controls and fifteen patients with GERD registered with sphinctometer.*

Mechanism of reflux	Control	GERD
TLESR	152 (63 %)	197 (37 %)
Swallow related TLESR ≥10 sec	20 (8 %)	77 (14 %)
Swallow induced LESR	26 (11 %)	108 (20 %)
LES pressure drift	11 (5 %)	31 (6 %)
Absent LES pressure	4 (1 %)	42 (8 %)
Abdominal strain	8 (3 %)	75 (14 %)
Unknown, other	22 (9 %)	6 (1 %)
Total	243 (100%)	536 (100%)

Figure 3. *Twenty four hour continuous registration of TLESR (n/h) in 15 patients with GERD (rhombs) and 10 control subjects (squares).*



DISCUSSION

Ambulatory 24 hour sphinctometer recording of LES pressure has shown diurnal variations with higher LES pressure during the supine period compared to upright periods both in controls and patients with GERD. In the upright periods the frequency of TLESRs was significantly lower in GERD patients compared to controls. This is the first report on prolonged ambulatory TLESR recording with the sphinctometer in controls and GERD patients. We confirm the results of others (4-6) using 24 hr ambulatory sleeve recording that TLESRs are the major mechanism of reflux in ambulatory healthy subjects and in patients with GERD.

LES pressure showed a diurnal pattern with increases during the evening resulting in higher LES pressure in the supine nighttime period compared to upright periods. Identical diurnal patterns have been described in studies with water-perfused sleeve manometry in recumbent asymptomatic subjects and patients with reflux esophagitis (1,2). LES pressure is influenced by body position. LES pressure was found to be higher in horizontal position compared to vertical position (19). However, other investigators did not observe an influence of body position on LES pressure (20,21). Sleep may also increase LES pressure, with pressure depending on the stage of sleep (22). Our twenty-four hour pressure profiles suggest a diurnal variation in LES pressure with elevated LES pressure during nighttime compared to daytime, although the influence of body position and sleep in our study cannot be separated. An elevated LES pressure at nighttime/supine position strengthens the barrier at the esophagogastric junction and therefore prohibits gastroesophageal reflux.

The frequency of TLESRs varied markedly during the day. Postprandially the number of TLESRs increased, resulting from gastric distention induced by the meal and from meal composition. During the supine period the frequency of TLESRs was significantly lower compared to upright periods. Body posture influences the frequency of TLESRs with significantly lower numbers of TLESRs in the supine position compared to upright/vertical body position (19-21). TLESRs are also inhibited by sleep with a diminished TLESR frequency especially during sleep stage 4 compared to the awake state (22). The differences in TLESR frequency we observed between day and night may therefore not only result from differences in body position but also from sleep. Because we did not register sleep by electroencephalogram, we cannot differentiate whether the changes in TLESRs results from supine position per se or from sleep.

Results on TLESRs obtained with ambulatory recordings are in line with data from stationary esophageal manometry using a water-perfused sleeve device (1,2). Studies comparing sleeve with sphinctometer technique have shown that the sphinctometer slightly underestimates TLESR frequency compared to the sleeve (15,16). During low LES pressure spontaneous LES relaxations are not scored because they do not meet the criteria for TLESR (13). In the present study we have included for TLESR analysis the tracings with low (<5 mmHg) LES pressure. Although we analyzed sphinctometer tracings with low LES pressure with adjusted criteria (16), thereby increasing the number of TLESRs scored, the frequency of TLESRs in reflux patients in our study (2.2 ± 0.1/h for GERD; 3.2 ± 0.2/h for controls) are in the same range as the data obtained with other ambulatory studies. We have previously shown during simultaneous measurement that sphinctometer and sleeve correlate very well with respect to TLESR detection (16). Therefore results from studies obtained with these techniques can be compared and were found to be in the same range.

TLESR account for about half of the acid reflux episodes in patients with GERD. We have shown that the contribution of "absent LES pressure", "abdominal straining" and "reflux during swallow-induced LES relaxations" as reflux mechanisms for acid reflux is increased in patients with GERD. Van Herwaarden et al (6) reported that excess reflux in GERD patients with hiatus hernia compared to those without hiatus hernia is caused by mechanisms other than TLESR such as low LES pressure, swallow-associated normal LES relaxation, deep inspiration and straining. In the present study four of the ten patients with GERD had a hiatal hernia. In patients with a hiatal hernia 39% of all reflux episodes occurred during TLESRs while in patients without a hiatal hernia TLESRs were responsible for a much higher percentage of 58% of all reflux episodes. Mechanism of reflux may differ according to the type of reflux patient. On the one end are subjects with a sufficient gastroesophageal (normal LES pressure) barrier who may have reflux predominantly through TLESRs versus on the other end subjects with low LES pressure and a hiatal hernia in whom gastroesophageal reflux predominantly occurs through non-TLESR mechanism.

Acid reflux is higher in patients with GERD due to several factors: increased number of reflux episodes, increased postprandial frequency of TLESRs, increased frequency of TLESR associated with acid reflux, and prolonged duration of reflux episodes. In the present study patients with GERD did not have an excess rate of TLESRs compared to controls. The duration of reflux episodes was only slightly increased compared to controls. However, a significantly higher percentage of TLESRs in the GERD patients was associated with reflux. In addition, mechanisms of reflux other than TLESRs accounted for the excess reflux in patients with GERD.

Simultaneous ambulatory esophageal (LES) manometry and pH recording may help to clarify mechanisms of acid reflux. Studies with the sphinctometer may improve our knowledge on pathophysiology of reflux, LES function and mechanisms of action of medical and surgical interventions in GERD patients. Compared to the water-perfused sleeve system (4,23), the sphinctometer is a compact, low weight equipment to study LES profiles. It should, however, be noted that LES pressure output of the sphinctometer is lower compared to output of a water-perfused sleeve. The sphinctometer is therefore not the ideal instrument to determine absolute LES pressure (16).

In conclusion: prolonged 24 hour ambulatory recording of the LES by sphinctometer has revealed that 1) circadian LES pressure profiles exist with elevated LES pressure during the supine/sleep period in control subjects but not in GERD patients. 2) TLESR frequency has a circadian profile both in controls and GERD patients. 3) TLESR frequency is not increased in GERD patients vs controls. 4) TLESR are the major mechanism of acid reflux in controls and patients with GERD.

REFERENCES

1. Dent J, Dodds WJ, Friedman RH, Sekiguchi T, Hogan WJ, Arndorfer RC et al. Mechanism of gastroesophageal reflux in recumbent asymptomatic human subjects. J Clin Invest 1980; 65:256-267.
2. Dodds WJ, Dent J, Hogan WJ, Helm JF, Hauser R, Patel GK, et al. Mechanisms of gastroesophageal reflux in patients with reflux esophagitis. N Engl J Med 1982; 307:1547-1552.
3. Mittal RK, Holloway RH, Penagini R, Blackshaw LA, Dent J. Transient lower esophageal sphincter relaxation. Gastroenterology 1995; 109:601-610.
4. Schoeman MN, Tippett MD, Akkermans LM, Dent J, Holloway RH. Mechanisms of gastroesophageal reflux in ambulant healthy human subjects. Gastroenterology 1995; 108:83-91.
5. Penagini R, Schoeman MN, Dent J, Tippett MD, Holloway RH. Motor events underlying gastroesophageal reflux in ambulant patients with reflux oesophagitis. Neurogastroenterol Mot 1996; 8 131-141.
6. Van Herwaarden M, Samsom M, Smout AJPM. Excess gastroesophageal reflux in patients with hiatus hernia is caused by mechanisms other than transient LES relaxations. Gastroenterology 2000; 119:1439-1446.
7. Holloway RH, Kocyan P, Dent J. Provocation of transient lower esophageal sphincter relaxations by meals in patients with symptomatic gastroesophageal reflux. Dig Dis Sci 1991;36:1034-1049.
8. Penagini R, Bianchi PA. Effect of morphine on gastroesophageal reflux and transient lower esophageal sphinter relaxation. Gastroenterology 1997;113:409-414.
9. Holloway RH, Lyrenas E, Ireland A, Dent J. Effect of intraduodenal fat on lower oesophageal sphincter functions and gastro-oesophageal reflux. Gut 1997;40:449-453.
10. Mittal RK, McCallum RW. Characteristics and frequency of transient relaxations of the lower esophageal sphincter in patients with reflux esophagitis. Gastroenterology 1988;95;593-599.
11. Penagini R, Mangano M, Bianchi PA. Effect of increasing the fat content but not the energy load of a meal on gastroesophageal reflux and lower oesophageal sphincter motor function. Gut 1998;42:330-333.
12. Wong WM, Lai KC, Hui WM, Hu WHC, Huang JQ, Wong NYH, Xia HHX, Chan OO, Lam SK, Wong BCY. Pathophysiology of gastroesophageal reflux diseases in Chinese – Role of transient lower esophageal sphincter relaxation and esophageal motor dysfunction. Am J Gastroenterol 2004;99:2088-2093.
13. Trudgill NJ, Riley SA. Transient lower esophageal sphincter relaxations are no more frequent in patients with gastroesophageal reflux disease than in asymptomatic volunteers. Am J Gastroenterol 2001;96:2569-2574.
14. Gotley DC, Barham CP, Miller R, Arnold R, Alderson D. The sphinctometer: a new device for measurement of lower oesophageal sphincter function. Br J Surg 1991; 78:933-935.
15. Pehlivanov N, Liu J, Arora T, Yamamoto Y, Mittal RK. Lower esophageal sphincter monitoring with sphinctometer: in vitro and in vivo studies. Am J Physiol 1999; 277:G577-G584.
16. Straathof JWA, Lüchtenborg M, Lamers CBHW, Masclee AAM. Comparison of two techniques for lower esophageal sphincter manometry: sleeve and sphinctometer. Neurogastroenterol Motil 2004; 16:265-268.
17. Trudgill NJ, Riley SA. Monitoring the lower esophageal sphincter - sphinctometer or sleeve. Neurogastroenterol Motil 1999; 11:173-178.
18. Holloway RH, Penagini R, Ireland AC. Criteria for objective definition of transient lower esophageal sphincter relaxation. Am J Physiol 1995; 268:G128-G133.

19. Freidin N, Mittal RK, McCallum RW. Does body posture affect the incidence and mechanism of gastro-oesophageal reflux? Gut 1991;32:133-136.
20. Ireland A, Dent J, Holloway RH. The role of head position in the postural control of transient lower oesophageal sphincter relaxations and belching. Gullet 1992;2:81-84.
21. Ireland A, Dent J, Holloway RH. Preservation of postural control of transient lower oesophageal sphincter relaxations in patients with reflux oesophagitis. Gut 1999;44:313-316.
22. Freidin N, Fisher MJ, Taylor W, Boyd D, Suratt P, McCallum RW, Mittal RK. Sleep and nocturnal acid reflux in normal subjects and patients with reflux oesophagitis. Gut 1991; 32:1275-1279.
23. Van Herwaarden MA, Samsom M, Akkermans LMA, Smout AJPM. Prolonged recording of oesophageal and lower oesophageal sphincter pressure using a portable water-perfused manometric system. Neurogastroenterol Mot 2001;13:111-119.

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ESOPHAGEAL BODY MOTILITY DURING ACID REFLUX EPISODES IN PATIENTS WITH REFLUX DISEASE

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ABSTRACT

Background: Esophageal body motility contributes to clearance of acid. Little is known about esophageal motility within acid reflux episodes during 24 hour ambulatory recording in patients with gastroesophageal reflux disease (GERD).

Methods: We studied esophageal motility during reflux episodes (ambulatory 24 hour esophageal pH- and manometry) in 89 patients with GERD and 15 healthy controls. Subjects were divided into subgroups based on acid exposure time.

Results: Amplitude and duration of esophageal contractions were not significantly different between the groups with normal, mild, moderate and severe reflux. The number of distal esophageal contractions required to raise esophageal pH above 4 was identical in the reflux subgroups during upright reflux, but was significantly ($p<0.05$) different during supine reflux. The frequency of peristaltic waves was significantly ($p<0.001$) decreased in subjects with severe acid reflux both upright and supine.

Conclusions: Esophageal body motility is not impaired during reflux episodes in GERD patients. In subjects with severe acid reflux, the clearance of acid is diminished as consequence of a significant reduction in the frequency of peristaltic waves.

INTRODUCTION

Gastroesophageal acid reflux predominantly occurs during transient lower esophageal sphincter (LES) relaxations or when LES pressure is very low.(1) Other factors involved in the pathogenesis of reflux disease are gastric emptying and composition of gastric refluxate.(2) The duration of a reflux episode is determined by the clearance of acid from the esophagus. Acid clearance is dependent on esophageal body motility and salivary buffering.(2,3)

Previous studies using stationary manometry indicate that esophageal body motility is abnormal in patients with gastroesophageal reflux disease (GERD) (4-10). Abnormalities that have been reported include: reduced amplitude of distal esophageal contractions, reduced velocity of peristalsis and higher percentage of uncoordinated contractions. These motor abnormalities are referred to as "ineffective esophageal motility" (IEM) (5,8,10,11).

In order to obtain a better insight into the relation between esophageal peristalsis and reflux, esophageal motility should be investigated more specifically within the time frame of a reflux episode. Ambulatory solid state manometry and pH metry have the advantage over stationary manometry that prolonged, 24 hour recording is feasible and thereby will more closely reflect the (patho)physiology of daily life.

Aim of our study was to evaluate the esophageal body motor events that contribute to esophageal acid clearance in reflux patients with varying severity of reflux disease.

MATERIALS AND METHODS

Subjects

Between 1997 and 2002 eighty nine patients referred to the motility unit of the department of Gastroenterology of the Leiden University Medical Center for 24 hr pH

monitoring agreed to undergo combined ambulatory 24 hr esophageal pH and manometry recording. The study populations consisted of patients with previously documented endoscopic esophagitis, according to the criteria of Savary and Miller12: twenty-nine patients with esophagitis grade I, twenty-five patients with esophagitis grade II, five patients with esophagitis grade III, four patients with esophagitis grade IV; (all patients with esophagitis grade I-IV: mean age 48 yr; range 20-75 yr; 34M; 29F) In twenty-six patients no erosive esophagitis was found: grade 0 (mean age 49 yr; range 20-75 yr; 14M; 12F). The control group consisted of fifteen healthy subjects (mean age 30 yr; range 19-56 yr; 8M; 7F). These subjects were free of reflux symptoms and were not on chronic medication. Informed consent was obtained from each individual. The study had been approved by the Ethics Committee of the Leiden University Medical Center.

Ambulatory 24 hour esophageal pH- and manometry

In all subjects esophageal manometry (water-perfusion system) was performed to determine the position (upper margin) of the LES. Thereafter a catheter containing three solid-state pressure sensors at 5 cm distance from each other and a glass pH electrode catheter were positioned in the esophagus with the distal pressure sensor and the tip of the pH electrode 5 cm above the LES. Esophageal pressure was recorded at 5, 10, 15 cm above the upper margin of the LES. Both catheters were connected to a portable digital recorder (Microdigitrapper, Medtronic, Denmark).

All studies were performed under ambulatory conditions on outpatient basis and each person was encouraged to follow her/his daily routine during registration. Intake of food and drinks with pH below 4 was restricted. During the study, periods of retrosternal pain or heartburn, eating and drinking, and supine position were registered in a diary. Upon completion of the 24-hour monitoring, all recorded data were transferred to a personal computer and stored for later analysis.

Data analysis

Selection of subjects by grade of esophagitis may have become less accurate since nowadays many patients have been treated with acid inhibitory drugs when endoscopy is performed. By then, endoscopic signs of esophagitis may have regressed or even disappeared. Therefore we considered it more appropriate to divide reflux patients into subgroups based on results of 24 hour pH-metry.

Analyses were done for upright and supine reflux separately. For upright reflux, the duration of time with esophageal pH<4 of 0-4% was defined as normal acid reflux (n=40, controls and patients); 4-8% pH<4 as mild upright acid reflux (n=21); 8-12% pH<4 as moderate upright acid reflux (n=17) and >12% as severe upright acid reflux (n=26).13 For supine reflux, acid exposure time below 1.2% was defined as supine reflux in the normal range (n=20), from 1.2% to 10% as mild-moderate supine reflux (n=26) and >10% as severe supine reflux (n=26).13Thirty two subjects had no acid reflux in supine position (time pH<4 when supine ≤ 0.1%) and were not included in the calculations on supine reflux.

An acid reflux episode was defined as a sudden fall in esophageal pH to below 4 with a duration of at least five seconds including the last set of peristaltic contractions that raised the pH to above 4. In all subjects the episodes of acid reflux were marked and based on the diary divided in upright or supine periods. The data were processed by automated analysis

(Multigram 6.31, Medtronic, Denmark). The following parameters of acid reflux were measured: total duration of all reflux episodes (min), number of reflux episodes, mean duration of reflux episodes (min), duration of longest reflux episode (min) and number of reflux episodes lasting longer than five minutes.

Esophageal pressure rises with an amplitude of at least 20 mmHg above the baseline and a duration between 1.0 and 5.0 seconds were recognized as a contraction. All other pressure variations were classified as artifacts or regarded as an unclassified activity. In case of multiple-peaked contractions, the amplitude of the second peak has to be at least 50% of the amplitude of the main peak, the trough-to-peak duration had to be at least 0.5 seconds and the trough had to be 15% of the main peak. During reflux episodes we obtained from each pressure channel: total number of contractions, frequency of contractions (contractions/min), amplitude (mmHg) and duration (sec) of esophageal contractions.

The computer program categorized each contraction as either peristaltic, simultaneous or non-transmitted. Contractions were considered peristaltic when the onset at the proximal esophageal recording site is more than 0.3 but less than 5.0 sec before the contraction onset in the consecutive pressure channel. Contractions in consecutive channels were categorized as simultaneous when the peak interval was less than 0.3 seconds. Non-transmitted contractions occurred in one channel only. A peristaltic wave was defined as a group of coordinated contractions. The frequency of peristaltic waves per reflux episode was measured. Esophageal motility was divided into effective and ineffective peristalsis. Effective peristalsis was defined as complete (proximal, mid and distal contractions) peristalsis with adequate contraction amplitude (≥30 mmHg). Ineffective peristalsis was defined as complete peristalsis but inadequate contraction amplitude or incomplete peristalsis or non-peristaltic contractions. Data were analyzed for upright and supine periods separately. For all parameters, a mean value per registration period was calculated. The analyzed data were transferred to a database program for statistical evaluation.

Statistical analysis

Parameters with normal distributions were expressed as mean ± SEM. Other parameters were expressed as median. The Mann-Whitney U test for non-parametric data was used for statistical analysis to compare results between the groups of patients and the controls. Analysis of variance, followed by Student-Newman-Keuls analysis, was used to compare results between the different groups. Correlations were calculated using Spearman's rank correlation coefficient (Rs). The significance level was set at $p<0.05$.

RESULTS

Esophageal pH and motility in upright position

Subjects were divided into subgroups based on acid exposure time. The number of reflux episodes, duration of reflux episodes, duration of the longest reflux episode and number of reflux episodes >5 min increased significantly ($p<0.001$) with the severity of acid reflux (Table 1).

Parameters of distal esophageal body motility during reflux episodes are shown in Table 2. The total number of contractions per reflux episode, that is the number of contractions needed to raise esophageal pH to level above 4, was not significantly different between the four groups (Figure 1). Subjects with severe acid reflux had a significantly ($p<0.001$) lower frequency of distal esophageal contractions in the reflux episode (1.4 ± 0.1 contractions/min) than subjects with acid reflux in the normal range (2.1±0.1 contractions/min) and mild acid reflux (1.9 ± 0.2 contractions/min). The amplitude of contractions in the distal esophagus was slightly but significantly ($p<0.05$) lower in subjects with severe acid reflux (47 ± 2 mmHg) compared to normal acid reflux (56 ± 2 mmHg) but are considered in all groups to be adequate (amplitude ≥30 mmHg). The frequency of contractions (n/min) per reflux episode correlated inversely with the severity of acid reflux ($Rs = -0.40$; $p<0.0001$).

The efficacy of esophageal motility during reflux episodes in the upright period is shown in Table 3. The frequency of peristaltic waves during a reflux episode was significantly ($p<0.001$) lower in the subjects with severe acid reflux (1.4 ± 0.1 contractions/min) compared to subjects with acid reflux in the normal range (2.2 ± 0.1 contractions/min) and mild acid reflux (1.9 ± 0.2 contractions/min). Effective peristalsis during reflux episodes in the subjects with severe acid reflux (33 ± 3%) was significantly ($p<0.001$) lower compared to subjects with acid reflux in the normal range (46 ± 3%). An increasing esophageal acid exposure time correlated inversely ($Rs = -0.27$; $p=0.005$) with the percentage effective peristalsis.

Table 1. *Characteristics of esophageal pH-metry during upright position (mean ± SEM). Patients with gastroesophageal reflux disease and controls are divided in subgroups based on acid exposure time in the upright position during the 24 hour period. ^ denote significant (p<0.001) differences versus the normal group; # denote significant (p<0.001) differences versus the mild group; * denote significant (p<0.001) differences versus the moderate group.*

Subgroup (subjects)	Normal (n=40)	Mild (n=21)	Moderate (n=17)	Severe (n=26)
Time esophageal pH<4 (%), upright	0-4%	4-8%	8-12%	>12%
Reflux time (min)	26 ± 3	62 ± 7	88 ± 14	128 ± 13
Number of reflux episodes	19 ± 3	44 ± 9^	59 ± 18^	49 ± 6^
Duration of reflux episode (min)	1.7 ± 0.3	1.7 ± 0.2	1.9 ± 0.2	3.1 ± 0.4^#*
Duration of longest reflux episode (min)	3.7 ± 0.5	8.5 ± 1.6	16.4 ± 6.3^#	16.8 ± 2.7^#
Number of reflux episodes >5 min	0.3 ± 0.1	2.0 ± 0.7^	2.6 ± 0.7^	4.4 ± 0.7^#*

Table 2. *Characteristics of esophageal motility during reflux episodes in upright position. ^ denotes a significant (p<0.001) difference versus the normal group, # denotes a significant (p<0.001) difference versus the mild group, * denotes a significant (p<0.05) difference versus the normal group.*

Time esophageal pH<4, upright	Normal (0-4%)	Mild (4-8%)	Moderate (8-12%)	Severe (>12%)
Number of distal contractions / reflux episode	3.6 ± 0.7	3.0 ± 0.3	3.3 ± 0.5	3.6 ± 0.5
Frequency of distal contractions (N/min)	2.1 ± 0.1	1.9 ± 0.2	1.7 ± 0.2	1.4 ± 0.1^#
Amplitude of distal contractions (mmHg)	56 ± 2	51 ± 3	50 ± 3	47 ± 2*
Duration of distal contractions (sec)	2.3 ± 0.1	2.3 ± 0.1	2.1 ± 0.1	2.0 ± 0.1

Table 3. *Efficacy of esophageal motility during reflux episodes in upright position. Effective peristalsis is described as complete peristaltic waves with adequate contraction amplitude(>30 mmHg). Ineffective peristalsis is incomplete peristalsis or non-peristaltic contractions or contractions with inadequate amplitude. ^ denote significant (p<0.001) differences versus the normal group, # denotes a significant(p<0.001) difference versus the mild group.*

Time esophageal pH<4, upright	Normal (0-4%)	Mild (4-8%)	Moderate (8-12%)	Severe (>12%)
Frequency of peristaltic waves per reflux episode (N/min)	2.2 ± 0.1	1.9 ± 0.2	1.8 ± 0.1	1.4 ± 0.1^#
Effective peristalsis (%)	46 ± 3	45 ± 3	41 ± 4	33 ± 3^
Ineffective peristalsis (%)	54 ± 4	55 ± 3	59 ± 4	67 ± 3

Esophageal pH and motility in supine position

The results of supine esophageal pH-metry and motility during all supine reflux episodes are shown in Tables 4-6. The duration of reflux episodes, duration of longest reflux episodes and number of reflux episodes lasting more than 5 min increased significantly ($p<0.001$) with the severity of supine acid reflux (Table 4).

The total number of distal esophageal contractions per reflux episode in subjects with severe supine reflux (7.7 ± 2.5 contractions) was significantly ($p<0.05$) higher compared to subjects with normal supine reflux (3.6 ± 1.2 contractions; Table 5 and Figure 1). The frequency of distal esophageal contractions was significantly ($p<0.05$) lower in subjects with mild-moderate supine reflux (1.2 ± 0.2 contractions/min) or severe supine reflux (1.1 ± 0.4 contractions/min) compared to subjects with normal supine reflux (2.7 ± 0.5 contractions/min). The severity of supine reflux correlated significantly both with the number of distal esophageal contractions (Rs = 0.60; $p<0.05$) and the frequency of distal contractions during reflux episodes (Rs = –0.43; $p<0.001$). Amplitude and duration of distal contractions in the reflux episodes were not significantly different between the three subgroups (Table 5). This was true not only for distal esophageal motility but also for mid and proximal esophageal motility (data not shown). Thus, subject with severe supine acid reflux did not have a lower amplitude of distal esophageal contractions but needed more contractions to clear the acid while the contraction frequency also was significantly lower.

The frequency of peristaltic waves was significantly ($p<0.01$)lower in subjects with severe supine reflux (1.1 ± 0.4 waves/min) and mild-moderate supine reflux (1.2 ± 0.2 waves/min) compared to normal supine reflux (2.7 ± 0.5 waves/min). The percentage of effective peristalsis was not significantly different between subgroups of supine acid reflux (Table 6).

Table 4. *Characteristics of esophageal pH-metry during supine position (mean ± SEM). Only subjects with supine acid reflux were included. ^ denote significant (p<0.001) differences versus the normal group.*

Time esophageal pH<4, supine	Normal (n=20)(<1.2%)	Mild-moderate (n=26) (1.2-10%)	Severe (n=26)(> 10%)
Reflux time (min)	6.5 ± 3.0	24 ± 3.3	91.9 ± 13.9
Number of reflux episodes	9 ± 4	8 ± 2	22 ± 12
Duration per reflux episode (min)	1.6 ± 0.5	4.9 ± 0.9	12.8 ± 3.0^
Duration longest reflux episode (min)	2.0 ± 0.8	11.2 ± 2.1	50.0 ± 13.1^
Number of reflux episodes >5 min	0.3 ± 0.3	0.9 ± 0.1	3.0 ± 1.1^

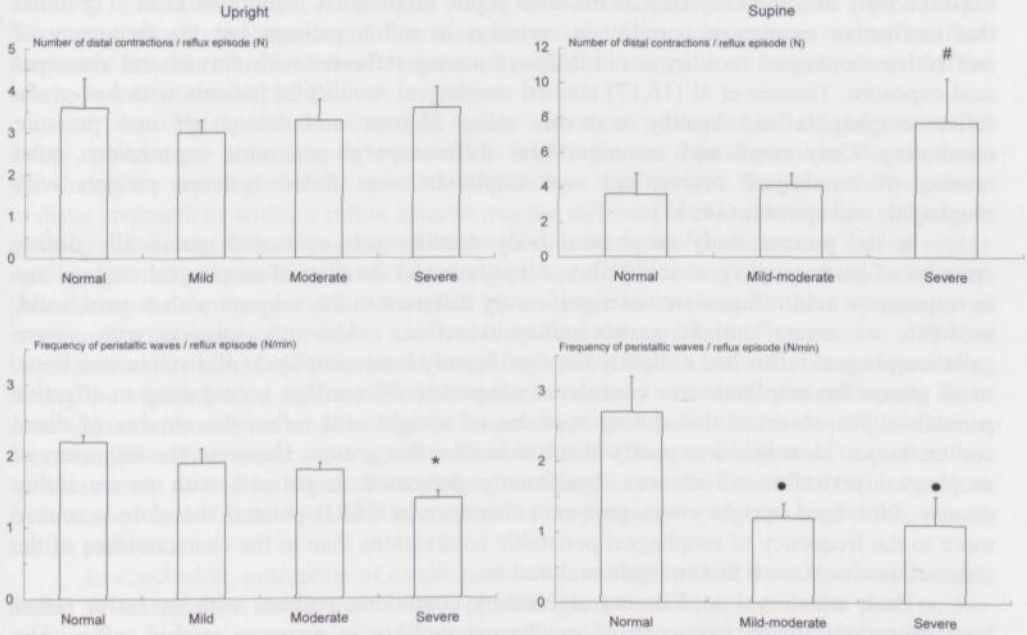
Table 5. *Characteristics of esophageal motility during reflux episodes in supine position. (mean ± SEM). ^ denote significant (p<0.05) differences versus the normal group*

Time esophageal pH<4	Normal (<1.2%)	Mild-moderate (1.2-10%)	Severe (>10%)
Number of distal contractions/reflux episode	3.6 ± 1.2	4.1 ± 0.7	7.7 ± 2.5^
Frequency of distal contractions (N/min)	2.7 ± 0.5	1.2 ± 0.2^	1.1 ± 0.4^
Amplitude of distal contractions (mmHg)	56 ± 8	50 ± 3	51 ± 3
Duration of distal contractions (sec)	2.3 ± 0.2	2.3 ± 0.2	2.7 ± 0.1

Table 6. *Efficacy of esophageal motility during reflux episodes in supine position. Effective peristalsis is described as complete peristaltic waves with adequate contraction amplitude (>30 mmHg). Ineffective peristalsis is incomplete peristalsis or non-peristaltic contractions or contractions with inadequate amplitude. ^ denote significant (p<0.01) differences versus the normal group.*

Time esophageal pH<4	Normal (0-1.2%)	Mild-moderate (1.2-10%)	Severe (>10%)
Frequency of peristaltic waves per reflux episode (N/min)	2.7 ± 0.5	1.2 ± 0.2^	1.1 ± 0.4^
Effective peristalsis (%)	37 ± 8	30 ± 4	37 ± 3
Ineffective peristalsis (%)	63 ± 8	70 ± 4	63 ± 3

Figure 1. *Number of distal esophageal contractions required to raise pH to a level above 4 in subjects with varying severity of reflux for upright reflux (left upper panel) and supine reflux (right upper panel). Frequency of peristaltic waves (n/min) during a reflux episode in subjects with varying severity of reflux for upright reflux (left lower panel) and supine reflux (right lower panel).*
*upright * $p<0.01$ severe vs mild and normal*
supine # $p<0.05$ severe vs normal and mild-moderate
supine • $p<0.01$ severe and mild-moderate vs normal



DISCUSSION

Esophageal motor dysfunction consisting of failed or hypotensive peristalsis may lead to impaired volume clearance of acid refluxate.(14) The severity of reflux esophagitis is related not only to the frequency but also to the duration of gastroesophageal reflux episodes (6,7). Previous studies indeed have shown that in patients with gastroesophageal reflux disease esophageal motility is impaired (4-11) Esophageal dysmotility is associated with more severe reflux symptoms and failure of medical treatment. On the other hand, reflux parameters or grade of esophagitis do not significantly differ between patients with or without dysmotility (15) Kahrilas et al (4) demonstrated that esophageal peristaltic function is impaired only in a minority of patients with peptic esophagitis. Vinjirayer et al (11) found that ineffective esophageal motility is common in reflux patients but the frequency of ineffective esophageal motility is not different among refluxers with normal and abnormal acid exposure. Timmer et al (16,17) studied esophageal motility in patients with low-grade reflux esophagitis and healthy controls, using 24-hour ambulatory pH and pressure monitoring. Only small and non-significant differences in peristaltic contractions, total number of esophageal contractions and amplitude were found between patients with esophagitis and controls (16, 17).

In the present study esophageal body motility was evaluated specifically during episodes of gastroesophageal acid reflux. Amplitude and duration of esophageal contractions in response to acid reflux were not significantly different in the subjects with normal, mild, moderate or severe upright gastroesophageal reflux. Although subjects with severe gastroesophageal reflux had a slightly but significantly lower amplitude of distal contractions, in all groups the amplitude was considered adequate (>30 mmHg), contributing to effective peristalsis. We observed that during episodes of upright acid reflux the number of distal contractions to clear acid was nearly identical in all reflux groups. However, the frequency of esophageal peristaltic waves was significantly decreased in patients with severe reflux disease. Prolonged upright esophageal acid clearance in GERD patients therefore is related more to the frequency of esophageal peristaltic contractions than to the characteristics of the contractions itself, such as amplitude or duration.

Thus, when evaluated during ambulatory conditions, patients with excessive reflux have alterations in the frequency of esophageal motility in response to acid reflux. The mechanism underlying the impaired distal motor response to reflux is not clear. Altered esophageal sensitivity may be a mechanism underlying the reduced peristalsis. Subjects with frequent heartburn but without esophageal erosions have a lower threshold for esophageal sensation and pain (18). Sensory thresholds of patients with excessive reflux or Barrett esophagus however are significantly higher compared to patients with non-erosive GERD (19,20). Visceral sensitivity is affected by age. Older GERD patients have reduced sensations to chemical or mechanical stimuli despite increased acid exposure (21,22). In our study the age of patients with severe reflux was not different from that of patients with less pronounced reflux.

Disordered peristaltic activity does not improve after healing of esophagitis (23,24). It has been suggested that impaired esophageal motility in reflux esophagitis is a consequence of esophageal inflammation but others have also pointed to impaired motility as a pre-existing factor in the pathogenesis of reflux. Our observations provide additional information because we focused on effective esophageal motility. We have clearly shown that under

physiological conditions peristaltic function in subjects with mild to severe upright acid reflux is not different from the group with normal reflux. The frequency of esophageal contractions was significantly decreased in subjects with severe acid reflux, but the amplitude of contractions was not significantly affected.

During the supine reflux periods no differences in amplitude or duration of esophageal contractions were shown between the groups with different acid exposure time. However, in patients with severe supine reflux the number of distal contractions required to clear the esophagus from acid was significantly higher compared to those with normal and mild-moderate reflux. Apart form that, the frequency of peristaltic waves was also reduced, resulting in markedly longer duration of reflux episodes in patient with severe supine reflux. Sleep may impair esophageal acid clearance, but arousal from sleep ensures normal swallow responses to acid reflux and characteristics of peristaltic contractions are not altered by the level of consciousness (25,26).

Comparing the results of upright reflux with those of supine reflux, the number of reflux episodes per hour in upright position is higher than in supine position. We observed that during the supine period the episodes lasted longer than upright. However, the frequency of distal contractions within a reflux episode was not different between the upright and supine position. Characteristics of esophageal contractions and peristaltic sequences during supine and upright reflux episodes did not show significant differences. Prolonged acid clearance time during the supine period is not caused by an impaired esophageal motor response, but more contractions/peristaltic waves are needed to clear the acid load. Timmer et al (17) also found that supine reflux episodes lasted longer in patients than in controls, but paradoxically, during the supine period reflux induced esophageal activity consisted of significantly more contractions with a higher amplitude and a longer duration in patients with esophagitis than in controls. This observation once again indicates that the longer duration of supine reflux episodes is not caused by impaired esophageal motility but results from other factors such as an increased acid volume load or a latency of the esophageal response to acid reflux during supine periods.

In conclusion, evaluation of esophageal body motility in GERD patients and controls during reflux episodes under ambulatory, physiological conditions has shown that: neither amplitude, nor duration nor velocity of esophageal peristalsis is affected both for upright and supine reflux episodes the frequency of distal esophageal contractions is significantly reduced in patients with severe acid reflux during upright reflux episodes the number of contractions to clear acid is constant among various reflux subgroups whereas during supine reflux episodes the number is significantly higher in patients with severe acid reflux.

REFERENCES

1. Dent J, Holloway RH, Toouli J, Dodds WJ. Mechanisms of lower esophageal sphincter incompetence in patient with symptomatic gastroesophageal reflux. Gut 1988;29:1020-1028.
2. Castell DO, Murray JA, Tutuian R, Orlando RC. Arnold R. Review article: the pathophysiology of gastro-esophageal reflux disease – oesophageal manifestations. Aliment Pharmacol Ther 2004;20:14-25.
3. Helm JF, Dodds WJ, Pelc LR, Palmer DW, Hogan WJ, Teeter BC. Effect of esophageal emptying and saliva on clearance of acid from the esophagus. N Engl J Med 1984;310:284-288.
4. Kahrilas PJ, Dodds WJ, Hogan WJ, Kern M, Arndorfer RC, Reece A. Esophageal peristaltic dysfunction in peptic esophagitis. Gastroenterology 1986;91:897-904.
5. Leite LP, Johnston BR, Barrett J, Castell JA, Castell DO. Ineffective esophageal motility (IEM): the primary finding in patients with nonspecific esophageal motility disorder. Dig Dis Sci 1997;42:1859-1865.
6. Kasapidis P, Xynos E, Mantides A, Chrysos E, Demonakou M, Nikolopoulos N, Vassilakis JS. Differences in manometry and 24-H ambulatory pH metry between patients with and without endoscopic or histological esophagitis in gastro oesophageal reflux disease. Am J Gastroenterol 1993;88:1893-1899.
7. Coenraad M, Masclee AAM, Straathof JWA, Ganesh S, Griffioen G, Lamers CBHW. Is Barrett's esophagus characterized by more pronounced acid reflux than severe esophagitis? Am J Gastroenterol 1998;93:1068-1072.
8. Ho S-C, Chang C-S, Wu C-Y, Chen G-H. Ineffective esophageal motility is a primary motility disorder in gastroesophageal reflux disease. Dig Dis Sci 2002;47:652-656.
9. Achem AC, Achem SR, Stark ME, DeVault KR. Failure of esophageal peristalsis in older patients: association with esophageal acid exposure. Am J Gastroenterol 2003;98:35-39.
10. Fouad YM, Katz PO, Hatlebakk JG, et al. Ineffective esophageal motility: the most common motility abnormality in patients with GERD-associated respiratory symptoms. Am J Gastroenterol 1999;94:1464-1467.
11. Vinjirayer E, Gonzalez B, Brensinger C, Bracy N, Obelmejias R, Katzka DA, Metz DC. Ineffective motility is not a marker for gastroesophageal reflux disease. Am J Gastroenterol 2003;98:771-776.
12. Savary M and miller G. The esophagus. Handbook and atlas of endoscopy. Solothurn, Switzerland: Gassmann A.G. 1978:135-139.
13. Masclee AAM, de Best ACAM, de Graaf R, Cluysenaer OJJ, Jansen JBMJ. Ambulatory 24-hour pH-metry in the diagnosis of gastroesophageal reflux disease. Scand J Gastroenterol 1990;25:225-230.
14. Kahrilas PJ, Dodds WJ, Hogan WJ. Effect of peristaltic dysfunction on esophageal volume clearance. Gastroenterology 1988;94:73-80.
15. Fibbe C, Layer P, Keller J, Strate U, Emmermann A, Zornig C. Esophageal motility in reflux disease before and after fundoplication: a prospective, randomized , clinical and manometric study. Gastroenterology 2001;121:5-14.
16. Timmer R, Breumelhof R, Nadorp JHSM, Smout AJPM. Esophageal motility in low-grade reflux esophagitis, evaluated by stationary and 24-hour ambulatory manometry. Am J Gastroenterol 1993;88:837-841.
17. Timmer R, Breumelhof R, Nadorp JHSM, Smout AJPM. Oesophageal motor response to reflux is not impaired in reflux oesophagitis. Gut 1993;34:317-320.
18. Rodriguez-Stanley S, Robinson M, Earnest DL, Greenwood-Van Meerveld B, Miner PB. Esophageal hypersensitivity may by a major cause of heartburn. Am J Gastroenterol 1999;94:628-631.

19. Winwood PJ, Mavrogiannis CC, Smith CL. Reduced sensitivity to intraoesophageal acid in patients with reflux induced strictures. Scan J Gastroenterol 1993;28:109-112.
20. Trimble KC, Pryde A, Heading RC. Lowered oesophageal sensory thresholds in patients with symptomatic but not excess gastro-oesophageal reflux: evidence for a spectrum of visceral sensitivity in GORD. Gut 1995;37:7-12.
21. Lasch H, Castell DO, Castell JA. Evidence for diminishing visceral pain with aging: studies using graded intraesophageal balloon distention. Am J Physiol 1997;272:G1-G3.
22. Fass R, Pulliam G, Johnson C, Garewal HS, Sampliner RE. Symptom severity and oesphageal chemosensitivity to acid in older and young patients with gastro-oesophageal reflux. Age and aging 2000;29:125-130.
23. McDougall NI, Mooney RB Ferguson WR, Collins JSA, McFarland RJ, Love AHG. The effect of healing oesophagitis on esophageal motor function as determined by oesophageal scintigraphy and ambulatory oesophageal motility/pH monitoring. Aliment Pharmacol Ther 1998;12:899-907.
24. Timmer R Breumelhof R, Nadorp JHSM, Smout AJPM. Oesophageal and gastroesophageal reflux before and after healing of reflux oesophagitis. A study using 24 hour ambulatory pH and pressure monitoring. Gut 1994;35:1519-1522.
25. Orr WC, Robinson MG, Johnson LF. Acid clearance during sleep in the pathogenesis of reflux esophagitis. Dig Dis Sci 1981;26:423-427.
26. Orr WC, Johnson LF, Robinson MG. Effect of sleep on swallowing, esophageal peristalsis and acid clearance. Gastroenterology 1984;86:814-819.

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PROSPECTIVE STUDY ON THE EFFECT OF LAPAROSCOPIC NISSEN FUNDOPLICATION ON REFLUX MECHANISMS

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Br J Surg 2001;88:1519-1524.

ABSTRACT

Background: Laparoscopic Nissen fundoplication effectively reduces acid reflux and reflux-symptoms. Little is known about the effect on reflux mechanisms especially on transient lower oesophageal sphincter relaxations (TLOSRs).

Methods: Twenty-seven patients were prospectively studied before and after laparoscopic Nissen fundoplication, by simultaneous recording of pH and lower oesophageal sphincter characteristics using sleeve manometry. In all of the twenty-seven patients the operation was judged successful, based on major improvement or resolution of reflux symptoms and acid reflux. Vagus nerve integrity was studied indirectly by the secretion of pancreatic polypeptide (PP) in response to insulin induced hypoglycaemia.

Results: After fundoplication basal LOS pressure significantly ($P<0.05$) increased from 13±1 mmHg to 22±1 mmHg. Laparoscopic Nissen fundoplication significantly ($P<0.05$) decreased the number of TLOSRs per hour in the fasting period from 2.5±0.5 TLOSR/h to 0.6±0.2 TLOSR/h and in postprandial period from 4.0±0.4 TLOSR/h to 1.3±0.3 TLOSR/h. The percentage of TLOSRs associated with reflux also decreased significantly ($P<0.05$), in the fasting period from 24±10% to 0±0% and postprandial period from 42±6% to 12±6%, respectively before and after fundoplication. Postoperatively the PP response was abnormal in three patients, pointing to vagus nerve dysfunction. Postoperative TLOSR frequency and LOS pressure were not different between patients with and without vagus nerve dysfunction.

Conclusions: Laparoscopic Nissen fundoplication significantly increases fasting and postprandial lower oesophageal sphincter pressure and significantly decreases the rate of TLOSRs. This results in a significant reduction of oesophageal acid exposure but postprandial LOS characteristics are preserved.

INTRODUCTION

Antireflux surgery is considered when patients with proven gastro-oesophageal reflux disease (GORD) are refractory to medical therapy (1). The Nissen fundoplication is the most commonly applied antireflux procedure. Since the introduction of laparoscopic techniques, antireflux surgery has received renewed attention. Minimally invasive antireflux procedures provide excellent symptom relief with rapid recovery and low postoperative morbidity, assuming sufficient surgical expertise (2).

Several studies have shown that the most prevalent mechanism of reflux in patients with GORD and in healthy subjects is the transient lower oesophageal sphincter relaxation (TLOSR) (3-5). Despite the fact that antireflux procedures are frequently performed, the mechanism of action of antireflux surgery is poorly understood. Previous studies on 'open' procedures have shown that Nissen fundoplication and partial (Belsey Mark IV) fundoplication reduce the frequency of TLOSR (6,7). Although much has been published about the outcome after laparoscopic Nissen fundoplication in terms of symptom relief (8) and reflux control (9), the effect of laparoscopic Nissen fundoplication on TLOSR has not been studied.

In the present study, patients with GORD were investigated prospectively before and six months after laparoscopic Nissen fundoplication with emphasis on TLOSR as the major mechanism of reflux. Oesophageal pH and sleeve manometry were performed under fasting conditions in response to a meal.

PATIENTS AND METHODS

Patients

Between January 1993 and December 1997 75 patients underwent laparoscopic Nissen fundoplication as primary antireflux procedure for GORD that was resistant to medical therapy. Twenty-seven of these patients agreed to participate in a prospective study evaluating the effect of the laparoscopic Nissen procedure on mechanisms of gastro-oesophageal reflux. All patients (11 male, 16 female; mean age 45 yr, range 21-72 yr) had symptoms of gastro-oesophageal reflux such as heartburn, regurgitation, retrosternal pain or dysphagia. GORD was documented in all patients by endoscopy and/or by ambulatory 24-hour pH monitoring. At this institution preoperative evaluation includes upper gastro-intestinal endoscopy, oesophageal manometry, 24-h ambulatory pH monitoring and vagus nerve function test. Postoperative evaluation, including oesophageal manometry, 24-h ambulatory pH metry and vagus nerve function test was performed 6 months after the operation.

Indications for antireflux surgery were the presence of reflux symptoms not responding sufficiently to prolonged medical treatment (more than 6 months) including proton pump inhibitors. Objective signs of reflux disease had to be present (endoscopic esophagitis and/or abnormal 24-h oesophageal pH measurements). The surgical procedure, laparoscopic Nissen fundoplication, was performed by one surgeon (J.R.). The operative technique entailed initial oesophageal mobilization and posterior hiatal repair. The gastric fundus was not routinely mobilized. The fundus was wrapped around the oesophagus, which was calibrated with a 42-Fr bougie within the oesophageal lumen, to enable construction of a floppy 360° fundoplication .

Mechanisms of gastro-oesophageal reflux were investigated in 27 patients before and 6-12 months after the operation. All patients were studied after an overnight fast. Medication was stopped at least 72 h before the study. Informed consent was obtained from each individual and the protocol had been approved by the local ethical committee.

Endoscopy

The severity of esophagitis was graded endoscopically according to the criteria of Savary and Miller. Preoperative endoscopy showed esophagitis grade 0 in six patients, grade I in eight patients, grade II in ten patients, grade III in three patients.

24 hour pH metry

We performed 24 hour ambulatory intra-oesophageal pH monitoring as described previously (10). Gastro-oesophageal reflux was considered pathological when the oesopgageal pH fell below 4 for 4.0 per cent or more of total recording time (10). Preoperative evaluation revealed abnormal 24 h pH measurements in 24 patients, in three patients reflux time was in the normal range but the patients had endoscopic signs of reflux disease (oesophageal pH below 4 for 0-4 per cent of the monitoring time).

Vagus nerve integrity

Vagus nerve integrity was measured indirectly by the secretion of plasma pancreatic polypeptide (PP) in response to insulin-induced hypoglycaemia (11,12). PP was measured by

a sensitive and specific radioimmunoassay (13). A peak increment in plasma PP below 47 pmol/l is considered to be compatible with vagus nerve dysfunction (11).

Manometric and pH technique

The manometry catheter consisted of a multilumen silicon tube (outer diameter 5.0 mm) incorporating a 6 cm long sleeve sensor (Dentsleeve Property, Belair, South Australia, Australia). The manometry catheter was introduced through the nose into the oesophagus and positioned so that the sleeve sensor straddled the LOS. A glass pH electrode (Ingold, Urdorf, Germany) positioned 5 cm above the upper margin of the LOS.

Study protocol

Experiments were started at 9 AM after an overnight fast. The subjects were studied in the upright position, sitting in a comfortable chair. They were not allowed to doze because of the effect of sleep on TLOSRs. Oesophageal manometry and pH were recorded simultaneously during a 30 min fasting period and for 180 min after a standard breakfast consisting of 400 ml Nutridrink (Nutricia, Zoetermeer, The Netherlands) containing 20 g protein, 26 g fat and 72 g carbohydrates (2520 kJ).

Lower oesophageal sphincter data and pH analysis

LOS tracings were analyzed for LOS resting pressure and LOS relaxations (LOSR) including TLOSR. TLOSRs were defined according to Mittal *et al.*(14) Gastro-oesophageal reflux episodes and the mechanisms of each reflux episode were scored using criteria described previously (7).

Statistical analysis

Data are expressed as mean ± SEM. Statistical analyses of TLOSR and reflux were performed using Wilcoxon's signed rank test and the Mann-Whitney U test. Differences in LOS pressure before and after operation and in response to meal ingestion were analyzed for statistical significance using multiple analysis of variance. A P-<0.05 was considered significant for all analyses.

RESULTS

In all of the 27 patients who participated in the study the operation was judged successful, based on major improvement or absence of reflux symptoms and gastro-oesophageal acid reflux. One patient had severe dysphagia after operation. This patient is included in the data analysis. After the postoperative studies she underwent reoperation during which the wrap was dismantled.

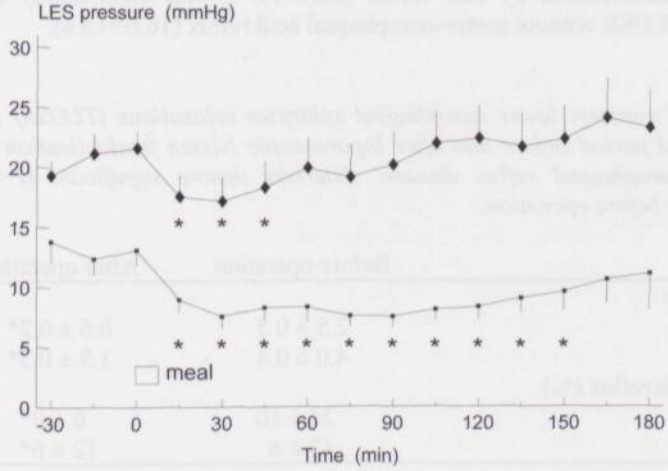
Vagus nerve integrity

Preoperatively, all patients had plasma PP peak increments in the normal range (above 47 pmol/l) before operation, with a mean value of 146±11 pmol/l. After fundoplication, the PP response was normal in all but three patients who had PP peak values indicative for vagus nerve dysfunction (47 pmol/l or less). None the less, the LOS pressure and TLOSR frequency of these three patients were not different from the other patients, who had no signs of vagus nerve dysfunction. The mean value of postoperative plasma PP peak increment of the patients with responses in the normal range was 121±10 pmol/l.

24 hour oesophageal pH metry

Compared to preoperative values total reflux time during 24-h oesophageal pH measurements decreased significantly from 11.7±2.3 (range 2.1-49.2)per cent to 0.8±0.3 (range 0-6.0)per cent.(P<0.01)

Figure 1. Mean (s.e.m.) *basal and postprandial lower oesophageal sphinter (LES) pressure at the gastro-oesophageal junction before (squares) and after laparoscopic Nissen fundoplication (diamonds) in 27 patients with gastro-oesophageal reflux disease. * P<0.05 versus basal value (before meal)(Manova test)l.*



Lower oesophageal sphincter pressure

After fundoplication basal LOS pressure before a test meal increased significantly from 13±1 mmHg to 22±1 mmHg (P<0.001) (Figure 1). Before operation, LOS pressure was significantly decreased (P<0.05) between 15 to 150 min after the start of the meal, reaching a minimum of 7±1 mmHg 30 min postprandially. After the operation, meal ingestion reduced LOS pressure between 15 to 45 min postprandially, reaching a minimum of 17±2 mmHg at 30 min. Postprandial LOS pressures after operation were significantly higher compared to preoperative values (P<0.001).

Transient lower oesophageal sphincter relaxations

TLOSRs were identified in all patients before operation. However, after laparoscopic Nissen fundoplication, TLOSRs were registered in only 21 of the 27 patients. In six subjects only spontaneous LOS relaxations with incomplete LOS relaxation were identified (Figure 2). All the postoperative TLOSR had a residual LOS pressure of 2 mmHg or less, according to the definition of TLOSR. A total of 76 spontaneous LOS relaxations which met all criteria for TLOSR apart from reaching the intragastric nadir pressure, were observed after laparoscopic Nissen fundoplication. Residual pressures during these relaxations varied from 3 to 28 mmHg. According to the definition these relaxations were not scored as TLOSR.

The total number of TLOSRs recorded in all subjects decreased after the operation from 47 to 11 in the fasting period and from 274 to 98 in the postprandial period. The number of TLOSRs per hour in the fasting period and after the meal decreased significantly (P<0.01; Table 1) postoperatively. Fundoplication not only reduced the number of TLOSRs but also significantly (P<0.05) decreased the percentage of TLOSRs associated with gastro-oesophageal acid reflux in the fasting and postprandial period from 24±10 and 42±6 per cent respectively before operation to 0±0 and 12±6 per cent respectively after operation.

The mean duration of TLOSR was 18.4±0.5 s before fundoplication and decreased significantly to 16.0±1.1 s after fundoplication (P<0.05). Preoperative and postoperative duration of TLOSRs accompanied by acid reflux (20.4±1.0 s) was significantly longer (P<0.001) than that of TLOSR without gastro-oesophageal acid reflux (16.6±0.5 s).

Table 1. *Frequency of transient lower oesophageal sphincter relaxations (TLOSR) in the fasting and postprandial period before and after laparoscopic Nissen fundoplication in 27 patients with gastro-oesophageal reflux disease. Asterisks denote significant (P<0.01) differences compared to before operation.*

	Before operation	After operation
TLOSR (number/hour)		
basal	2.5 ± 0.5	0.6 ± 0.2*
postprandial	4.0 ± 0.4	1.3 ± 0.3*
TLOSR associated with reflux (%)		
basal	24 ± 10	0 ± 0*
postprandial	42 ± 6	12 ± 6*

Mechanism of reflux

The total number of gastro-oesophageal reflux episodes was 284 before operation and 15 after operation (Table 2). The predominant mechanism of reflux was TLOSR: 43% before and 60% after fundoplication.

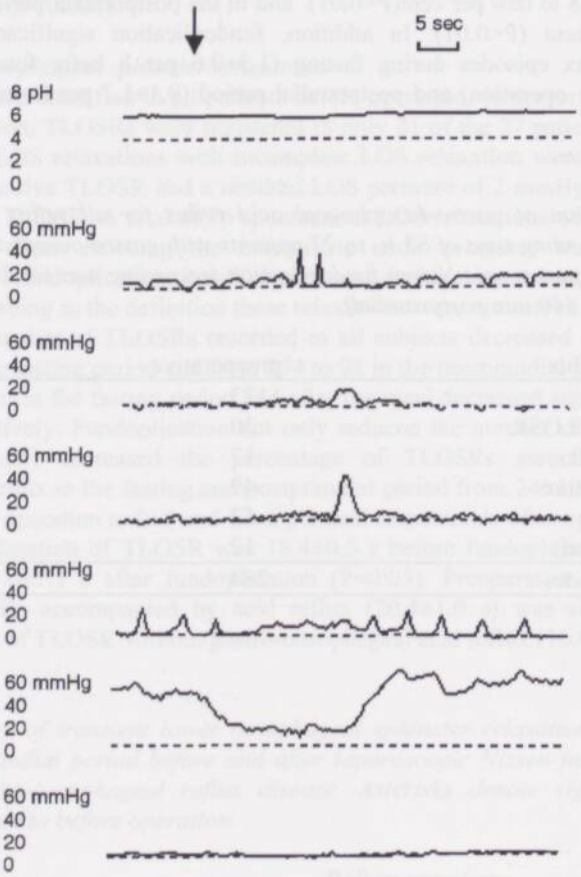
Gastro-oesophageal reflux

Fundoplication significantly reduced oesophageal acid exposure time in the basal period from 1.7±0.8 to 0±0 per cent($P<0.01$) and in the postprandial period from 11.7±3.7% to 0.03±0.02 percent ($P<0.01$). In addition, fundoplication significantly decreased the frequency of reflux episodes during fasting (1.3±0.6 per h befre fundoplication versus. 0.1±0.1 per h after operation) and postprandial period (9.1±1.7 per h versus 0.5±0.2 per h) ($P<0.01$).

Table 2. *Mechanism of gastro-oesophageal acid reflux for all reflux episodes observed during a total recording time of 85 h in 27 patients with gastro-oesophageal reflux disease before and after laparoscopic Nissen fundoplication (recording was for 190 min per patient: 30 min fasting and 160 min postprandial).*

Mechanism of reflux	preoperative	postoperative
TLOSR	123	9
Swallow induced LOSR	30	0
LOS pressure drift	17	1
Absent LOS pressure	49	0
Abdominal strain	53	0
Other (unexplained)	12	5
Total reflux episodes	284	15

Figure 2.*Tracing of an 'incomplete' transient lower oesophageal sphincter relaxation (arrow) in a patient after laparoscopic Nissen fundoplication. The tracings show (from top to bottom) oesophageal pH, pharyngeal pressure, three oesophageal body pressure channels, lower oesophageal sphincter (LOS) pressure and intra gastric pressure. The dotted line denotes intragastric pressure or pH 4. The LOS pressure does not decrease to intragastric pressure during a spontaneous LOS relaxation.*



DISCUSSION

Laparoscopic Nissen fundoplication effectively reduces gastro-oesophageal reflux (15,16). However the effect of laparoscopic surgery on reflux mechanisms had not been studied previously. This study has shown that the number of TLOSRs is decreased both in fasting and the postprandial state after the laparoscopic Nissen fundoplication. These results are consistent with findings in a previous study on the effect of open Nissen fundoplication on the rate of TLOSR (6). A study on the effect of partial fundoplication (Belsey Mark IV) on TLOSR also showed a postoperative reduction in the rate of TLOSR and a decrease in the percentage of TLOSR associated with gastro-oesophageal acid reflux (7).

The mechanism by which laparoscopic Nissen fundoplication decreases the number of TLOSR is not understood. Several factors may be involved. Distension of the proximal stomach, either by air insufflation, by insufflation of barostat bag or by meal ingestion, is a potent trigger for TLOSR. The frequency of TLOSR is related to the volume of the proximal stomach and the degree of fundic distention (17). After Nissen fundoplication postprandial relaxation of the proximal stomach is impaired; i.e. after meal ingestion the maximum volume of the proximal stomach is significantly smaller in patients with Nissen fundoplication than in non-operated patients with GORD or healthy controls (18,19). Therefore the decreased frequency of TLOSR after fundoplication may be related to impaired postprandial relaxation of the proximal stomach.

The vagus nerve is involved in the pathway through which TLOSR are triggered. Vagotomy and atropine decrease the frequency of TLOSR (20,21). After Nissen fundoplication vagus nerve dysfunction may occur in up to 30 per cent and lead to symptoms such as nausea, bloating or diarrhoea. Such symptoms were observed in the patients who participated in this study. There was evidence for postoperative vagus nerve dysfunction in only 3 of the 27 subjects. The reduction in frequency of TLOSR after the operation does not therefore appear to result from vagus nerve dysfunction.

After fundoplication TLOSR with complete LOS relaxation reaching intragastric pressure occurred in 21 patients. However, in six subjects only incomplete spontaneous LOS relaxations occurred. The incomplete TLOSRs have all the characteristics required for TLOSR apart from residual LOS pressure. Fundoplication not only influences the frequency of TLOSR but may also prohibit complete relaxation of the LOS during TLOSR or during swallowing.

Dysphagia is common in the early postoperative period and may be related to LOS dysfunction especially incomplete LOS relaxation. In a recently published randomized clinical trial of open versus laparoscopic Nissen fundoplication the relative risk of postoperative dysphagia was in favour of open surgery. but LOS relaxation was not evaluated (22). Apart from acid reflux TLOSR are also a mechanism for venting gas for the stomach, i.e. belching (23). An impaired ability to belch is common after antireflux surgery. Johnsson *et al* (24) noted that patients after 360° fundoplication with concomitant highly selective vagotomy did not exhibit TLOSR or common cavities in response to gastric distension. An absence of TLOSR may therefore be related to an inability to belch and may lead to gas bloat syndrome. The frequency of TLOSR decreased after After laparoscopic Nissen fundoplication in all of the present patients but TLOSRs, both complete and incomplete, still occurred; this may be relevant for the physiological venting of air from the stomach.

In conclusion, laparoscopic Nissen fundoplication significantly increased fasting and postprandial LOS pressure and significantly decreased the rate of TLOSR. It significantly decreased oesophageal acid exposure and reduced mechanisms of reflux, however, with preservation of postprandial LOS characteristics.

REFERENCES

1. Anvari M, Allen C, Borm A. Laparoscopic Nissen fundoplication is a satisfactory alternative to long-term omeprazole therapy. Br J Surg 1995; 82: 938-42.
2. McKernan JB, Champion JK. Minimally invasive antireflux surgery. Am J Surg 1998; 175: 271-6.
3. Dent J, Holloway RH, Toouli J, Dodds WJ. Mechanisms of lower oesophageal sphincter incompetence in patients with symptomatic gastrooesophageal reflux. Gut 1988; 29: 1020-8.
4. Dodds WJ, Dent J, Hogan WJ, Helm JF, Hauser R, Patel GK, et al. Mechanisms of gastroesophageal reflux in patients with reflux esophagitis. N Engl J Med 1982; 307: 1547-52.
5. Dent J, Dodds WJ, Friedman RH, Sekiguchi T, Hogan WJ, Andorfer RC, et al. Mechanism of gastroesophageal reflux in recumbent asymptomatic human subjects. J Clin Invest 1980; 65: 256-67.
6. Ireland AC, Holloway RH, Toouli J, Dent J. Mechanisms underlying the antireflux action of fundoplication. Gut 1993; 34: 303-8.
7. Masclee AAM, Horbach JM, Ledeboer M, Lamers CBHW, Gooszen HG. Prospective study of the effect of the Belsey Mark-IV fundoplication on reflux mechanisms. Scand J Gastroenterol 1998; 33: 905-10.
8. Bittner HB, Meyers WC, Brazer SR, Pappas TN. Laparoscopic Nissen fundoplication: operative results and short- term follow-up. Am J Surg 1994; 167: 193-8.
9. Mathew G, Watson DI, Myers JC, Holloway RH, Jamieson GG. Oesophageal motility before and after laparoscopic Nissen fundoplication. Br J Surg 1997; 84: 1465-9.
10. Masclee AAM, de Best ACAM, de Graaf R, Cluysenaer OJ, Jansen JBMJ. Ambulatory 24-hour pH-metry in the diagnosis of gastroesophageal reflux disease. Determination of criteria and relation to endoscopy. Scand J Gastroenterol 1990; 25: 225-30.
11. Jansen EH, Horbach JMLM, Klamer M, Jansen JBMJ, Hopman WPM, Gooszen HG, et al. Plasma pancreatic polypeptide response to insulin hypoglycaemia after Nissen fundoplication. Scand J Gastroenterol 1989; 24: (suppl 171): 9-12.
12. Masclee AAM, Lamers CBHW. Effect of endoscopic sclero-therapy of esophageal varices on vagus nerve integrity. J Hepatol 1994; 21: 724-9.
13. Lamers CBHW, Diemel CM, Leer van E, Leusen van R, Peetoom JJ. Mechanism of elevated serum pancreatic polypeptide concentrations in chronic renal failure. J Clin Endocrinol Metab 1982; 55: 922-6.
14. Mittal RK, Holloway RH, Penagini R, Blackshaw LA, Dent J. Transient lower esophageal sphincter relaxation. Gastroenterology 1995; 109: 601-10.
15. Gotley DC, Smithers BM, Rhodes M, Menzies B, Branicki FJ, Nathanson L. Laparoscopic Nissen fundoplication. 200 consecutive cases. Gut 1996; 38: 487-91.
16. Pursani KG, Sataloff DM, Zayas F, Castell DO. Evaluation of the antireflux mechanism following laparoscopic fundoplication. Br J Surg 1997; 84: 1157-61.
17. Boulant J, Mathieu S, D'Amato M, Abergel A, Dapoigny M, Bommelaer G. Cholecystokinin in transient lower oesophageal sphincter relaxation due to gastric distension in humans. Gut 1997; 40: 575-81.
18. Vu M, Straathof JWA, van der Schaar PJ, Arndt JW, Ringers J, Lamers CBHW, et al. Motor and sensory function of the proximal stomach in reflux disease and after laparoscopic Nissen fundoplication. Am J Gastroenterol 1999; 94: 1481-9.
19. Wijnhoven BPL, Salet GAM, Roelofs JMM, Smout AJPM, Akkermans LMA, Gooszen HG. Function of the proximal stomach after Nissen fundoplication. Br J Surg 1998; 85: 267-71.
20. Martin CJ, Patrikios J, Dent J. Abolition of gas reflux and transient lower esophageal sphincter relaxation by vagal blockade in the dog. Gastroenterology 1986; 91: 890-6.

21. Mittal RK, Holloway R, Dent J. Effect of atropine on the frequency of reflux and transient lower esophageal sphincter relaxation in normal subjects. Gastroenterology 1995; 109: 1547-54.
22. Bais JM, Bartelsman JFWM, Bonjer HJ, Cuesta MA, Go PMHYH, Klinkenberg-Knol EC, et al. Laparoscopic or conventional Nissen fundoplication for gastro-oesophageal reflux disease: randomised clinical trial. Lancet 2000; 355: 170-4.
23. Wyman JB, Dent J, Heddle R, Dodds WJ, Toouli J, Downton J. Control of belching by the lower oesophageal sphincter. Gut 1990; 31: 639-46.
24. Johnsson F, Holloway RH, Ireland AC, Jamieson GG, Dent J. Effect of fundoplication on transient lower oesophageal sphincter relaxation and gas reflux. Br J Surg 1997; 84: 686-9.

SUM

SUMMARY

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REFLUX MECHANISMS IN GERD:
Analysis of the role of transient lower esophageal sphincer relaxations.

The studies described in this thesis have dealt with the role of transient lower esophageal sphincter relaxations (TLESR) in gastroesophageal reflux disease (GERD). TLESR and other mechanisms of acid reflux have been studied in healthy volunteers, in patients with GERD and patients after antireflux surgery. We have successfully triggered TLESR by meal ingestion and by gastric distension. Secondly, we have investigated the effect of the several gastrointestinal peptides and neuro, endo or paracrine transmitters on lower esophageal sphincter (LES) characteristics.
We have employed two manometry techniques: the waterperfused Dentsleeve and the solid-state sphinctometer. The sphinctometer has been used especially to study mechanisms of reflux under ambulatory conditions. In addition mechanisms of esophageal body clearance were studied under ambulatory conditions.

Available data on the role of TLESR in GERD have been reviewed in the introduction, *chapter 1*. The frequency of TLESR, mechanisms of triggering TLESR and mechanisms of inhibition of TLESR and the influence of neural control were discussed. Clinically relevant aspects were highlighted. The concept that reflux patients have more TLESRs per time interval is not uniformly accepted. Perhaps other factors that permit reflux during a TLESR are more relevant. The LES is regulated by both neural and hormonal mechanism but especially neural factors are relevant for TLESR triggering.

In *chapter 2* we described mechanisms of acid reflux in 114 patients with reflux disease. The results were compared with data of 30 healthy control subjects. Basal fasting frequency of TLESR was not significantly different between patients without or with esophagitis and controls (range 2.1 – 3.8 TLESR/h). However postprandially the frequency of TLESR increased significantly more in controls (2.8 to 6.1 TLESR/h) than in patients with severe esophagitis (2.1 to 3.3 TLESR/h). In the postprandial state GERD patients with low LES pressure had significantly less TLESR compared to controls and to patients with intermediate and high LES pressure. TLESR are the predominant mechanism of reflux in healthy subjects (82%). In patients with GERD the mechanism of reflux is dependent on LES pressure. In patients with GERD who have a high LES pressure the main mechanism of reflux is reflux during TLESR (72%). In patients with low LES pressure gastroesophageal reflux does occur during TLESR (34%) but the majority of reflux episodes occur during low/absent LES pressure. It is concluded that: in GERD patients, frequency of TLESR in the fasting state is not different from controls and postprandially the frequency even is significantly reduced. Secondly in GERD patients significantly more TLESR are associated with reflux. Third, in GERD patients TLESR are the major mechanism of reflux but subtypes of refluxers exist (TLESR dominant vs. LES incompetence). And fourth LES pressure and not hiatus hernia or endoscope grade of esophagitis determine the reflux mechanism.

Gastric distension is a potent trigger for TLESR. In *chapter 3* we studied the occurrence of TLESR, common cavities (indicator for gas gastroesophageal reflux) and belching after standardized acute gastric distention by air insufflation in control subjects, patients with GERD and patients after fundoplication. Gastric distension provokes TLESR

but does not differentiate patients with GERD (3.1 TLESR/20 min) from controls (3.5 TLESR/20 min). In patients after fundoplication the frequency of TLESR (1.8 TLESR/20 min) was significantly reduced. Common cavities occurred mainly during relaxations of the LES. The main mechanism permitting common cavities was a TLESR (50-60%). After antireflux surgery intragastric insufflation of air revealed impaired belching capacity in two-third of the patients while half of them have symptoms in daily life.

The barostat technique was used to study continuous gastric distensions (*chapter 4*). Distension of the proximal stomach increases the frequency of TLESRs (2.1-3.5 TLESR/15 min), but adaptation occurs already after 15 min. In the second 15 min period the frequency was comparable with the basal frequency. The frequency of TLESR during distension is related to pressure and wall tension rather than to intragastric balloon volume.

In *chapter 5* we have shown that gastrin-17 decreases LES pressure when infused at physiological postprandial plasma levels. Gastrin-17 did not influence the occurrence of TLESR. However, gastrin increased gastroesophageal reflux (20 vs. 55 reflux episodes) and increased the percentage of TLESR associated with reflux (20 vs. 61%).

In *chapter 6* we have shown that in healthy subjects under fasting condition, somatostatin does not affect LES pressure or TLESR frequency (2.1 vs. 3.3 TLESR/h). During somatostatin infusion no postprandial decrease in LES pressure occurred; on the contrary, LES pressure gradually increased. After the meal the frequency of TLESR increased (2.6 vs. 4.3 TLESR/h). Somatostatin prevented not only the postprandial reduction in LES pressure but also the postprandial increase in TLESR frequency.

Bombesin the mammalian counterpart of gastrin releasing peptide is known for the fact that it increases LES pressure in healthy subjects. In *chapter 7* we investigated the effect of bombesin not only on LES pressure but also on TLESR. In patients with GERD and patients after fundoplication bombesin also significantly increases LES pressure. An important finding was that during bombesin-elevated LES pressure complete LES relaxations (TLESR) still occurred. The frequency of TLESR was not influenced by bombesin neither under basal conditions nor after gastric distension with air.

We have studied the effect of intravenous L-arginine, a NO donor, on LES characteristics in *chapter 8*. Ingestion of a carbohydrate-high fat meal significantly increased the frequency of TLESR (2.7 to 4.5 TLESR/h). L-arginine administration resulted in increasing plasma levels of L-citrulline indicating increased nitric oxide (NO) production. Neither under fasting nor under postprandial conditions did L-arginine significantly influence LES pressure TLESR or esophageal motility.

In *chapter 9* the sphinctometer was compared with the waterperfused sleeve. LES pressure and LES relaxation measured by sleeve and sphinctometer showed identical patterns. However, output of the sphinctometer was significantly lower than output of the sleeve. In healthy subjects with normal LES pressure the sphinctometer registers TLESR with results comparable to sleeve recording. However when the LES pressure is low the true number of TLESR is underscored by the sphinctometer. Adjustment of the criteria for

sphinctometer pressure readings improved identification of TLESR. When these criteria were applied, over 90% of TLESR measured by the sleeve were also classified as TLESR. On the other hand, TLESRs were missed by sleeve recording, while clearly present on a sphinctometer recording. Therefore, the sleeve is no longer the golden standard for TLESR registration.

In *chapter 10* we studied LES characteristics in healthy subjects and patients with GERD by ambulatory sphinctometer recording. We showed circadian LES pressure profiles with elevated LES pressure during the supine or sleep period. The frequency of TLESR was increased after meal ingestion and decreased during the supine period. In healthy controls and patients with GERD, TLESR were registered as the main mechanism of reflux (71 vs. 51 %). We concluded that the sphinctometer as low weight equipment deserves more attention and should be considered in the evaluation of LES characteristics in ambulatory setting. However the sphinctometer is not the ideal instrument to determine absolute LES pressure.

In *chapter 11* mechanisms of esophageal acid clearance were studied in patients with GERD and healthy volunteers. During reflux episodes the frequency of peristaltic contractions and waves was significantly reduced in patients with severe acid reflux. But the number of contractions to clear the esophagus of acidic content was nearly identical in the four subgroups (range 3.0-3.6 contractions per reflux episode). The characteristics of esophageal contractions like amplitude and duration are not significantly different in patients with severe acid reflux compared to patients with reflux in the normal range.

In *chapter 12* we have investigated the effect of laparoscopic Nissen fundoplication on reflux mechanisms in a prospective study. Laparoscopic Nissen fundoplication significantly increases LES pressure (13 to 22 mmHg) and significantly reduces the rate of TLESR (fasting: 2.5 to 0.6 TLESR/h; postprandially: 4.0 to 1.3 TLESR/h). Laparoscopic Nissen fundoplication significantly reduced esophageal acid exposure and reduced mechanisms of reflux, however with preservation of postprandial LES characteristics.

Table 1. *Factors that influence LES characteristics.*
≡ denotes no significant effect; ↑ increase; ↓ decrease.

	TLESR frequency	LES pressure
Meal	↑	↓
Gastric distension	↑	(↓)
Fundoplication	↓	↑
Gastrin	≡	↓
Bombesin	≡	↑
Somatostatin	≡	≡
Somatostatin + meal	≡	↑
L-arginine (NO-precursor)	≡	≡
L-arginine + meal	≡	≡

DISCUSSION

Aim of this thesis was to evaluate the role of TLESR in gastroesophageal reflux disease.

Meal ingestion increased the frequency of TLESR. Gastric distension plays an important role in this postprandial increase frequency of TLESR, since distension of the proximal stomach with air and distension with an intragastric bag both significantly increased the number of TLESR. Gastrointestinal peptides and neurotransmitters such as gastrin, somatostatin and bombesin and l-arginine did not influence TLESR frequency. However, these peptides did influence LES pressure. Gastrin decreased LES pressure at postprandial plasma levels, somatostatin inhibited the postprandial decrease of LES pressure and bombesin increased LES pressure. An intriguing finding was that during bombesin-elevated LES pressure, TLESR still occurred. These findings suggest that TLESR occur independent of LES pressure.

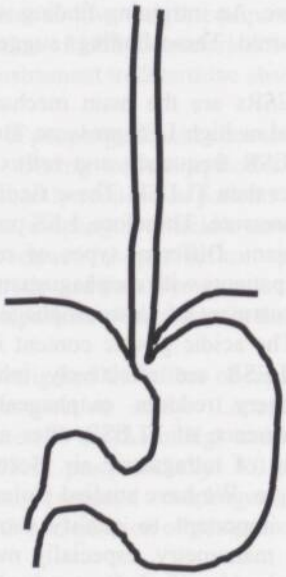
In healthy subjects TLESRs are the main mechanism of reflux as is the case in patients with GERD with normal or high LES pressure. But patients with low LES pressure have a significantly lower TLESR frequency and reflux in these patients predominantly occurs through mechanisms other than TLESR. These findings suggested that the mechanism of reflux is dependent on LES pressure. Therefore, LES pressure is an important predictor of reflux type and reflux mechanism. Different types of refluxers may exist: patients with TLESR predominant reflux and patients with esophagogastric junction incompetence.

The first choice in the treatment of gastroesophageal reflux is an anti-secretory drug like a proton pump inhibitor. The acidic gastric content is firmly affected, but esophageal motility is not influenced. TLESR are effectively inhibited by (laparoscopic Nissen) fundoplication. Antireflux surgery reduces esophageal acid exposure and decreases symptoms of GERD. The occurrence of TLESR after antireflux surgery is important to maintain the ability of belching of intragastric air. Secondly, inability of complete LES relaxation may result in dysphagia. We have studied patients with reflux disease before and after antireflux surgery. It is important to qualify esophageal motility before surgical intervention. Using esophageal manometry, especially measurement of LES pressure, the different types of refluxers can be identified. Customized care should be given to patients with GERD: medical therapy, surgical or endoscopic intervention.

Currently endoscopic antireflux procedures are performed more frequently. The first reports show mainly decrease in GERD symptoms and less dependency on proton pump inhibitor. The effect endoscopic antireflux procedures on LES characteristics, like TLESR and LES pressure has to be evaluated and compared with results of laparoscopic antireflux surgery.

Ambulatory monitoring of esophageal body motility shows only minor differences in amplitudes and duration of esophageal peristaltic contractions in patients with severe gastroesophageal reflux disease. Detailed information during acid reflux episodes showed a decreased frequency of peristaltic contractions and waves in patients with severe gastroesophageal reflux to clear the esophagus of acidic content. Ambulatory registration using the sphinctometer showed circadian profiles of TLESR and LES pressure. During supine period TLESR frequency is lower compared to upright period. Measurement of esophageal and LES motility may therefore be helpful to study motility disorders and follow up of intervention like surgical or endoscopic antireflux procedures.

In conclusion: we have studied esophageal motility especially TLESR and LES pressure in healthy subjects, patients with GERD and patients after laparoscopic Nissen fundoplication. The investigations have resulted in better understanding of functions of the esophagus especially the esophagogastric junction as barrier for gastroesophageal reflux. TLESR are spontaneous relaxations of the LES, which can be triggered by gastric distention. TLESR can also be inhibited, but absence or a pronounced reduction of these complete LES relaxations may negatively affect the safety "valve function" of the LES, which is venting of excess air.



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SAMENVATTING

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REFLUX MECHANISMEN IN GASTRO-OESOFAGEALE REFLUX ZIEKTE:
Analyse van de rol van spontane onderste slokdarm sfincter relaxaties.

Dit proefschrift beschrijft de rol van de transiente onderste slokdarm sfincter relaxaties (TLESR) in gastro–oesofageale refluxziekte. TLESR en andere mechanismen van zure reflux zijn bestudeerd in gezonde vrijwilligers en patiënten met refluxziekte en patiënten na anti-reflux chirurgie.
Wij hebben TLESRs succesvol gestimuleerd ten eerste met behulp van maaltijd innames en distensie van de proximale maag. Ten tweede hebben we het effect van enkele gastro-intestinale peptiden en neuro-, endo- of paracriene transmitters onderzocht op de onderste slokdarm sfincter karakteristieken.
Wij hebben gebruik gemaakt van twee manometrie technieken. De met water geperfundeerde Dentsleeve en de electronische sphinctometer. De sphinctometer is speciaal gebruikt voor het bestuderen van mechanismen van reflux onder ambulante condities. Tevens hebben wij gekeken naar de peristaltische klaring van de tubulaire slokdarm onder ambulante condities.

Beschikbare data over de rol van de TLESR in refluxziekte zijn beschreven in de introductie, *hoofdstuk 1*. De frequentie van TLESR, mechanismen om TLESR te stimuleren dan wel mechanismen om TLESR te onderdrukken en de invloed van neurale controle werden bediscussieerd. Klinisch relevante aspecten zijn benadrukt. De hypothese dat refluxpatiënten meer TLESR per tijdseenheid hebben is niet universeel geaccepteerd. De onderste slokdarm sfincter wordt gereguleerd door zowel neurale als hormonale mechanismen maar vooral neurale factoren zijn belangrijk voor het stimuleren van TLESR.

In *hoofdstuk 2* beschreven we de mechanismen van zure reflux in 114 patiënten met refluxziekte. De resultaten zijn vergeleken met de data van 30 gezonde vrijwilligers. De basale, nuchtere frequentie van TLESR was niet significant verschillend tussen patiënten met of zonder reflux-oesofagitis in vergelijking met controle patiënte (range 2.1- 3.8 TLESR/h). Maar postprandiale frequentie van TLESRs nam significant toe in de controle groep (2.8 tot 6.1 TLESR/h) in vergelijking met patiënten met ernstige reflux-oesofagitis (2.1 tot 3.3 TLESR/uur). Na een maaltijd hebben patiënten met een refluxziekte met een lage sfincterdruk een significant lager aantal TLESRs in vergelijking met de controles en in vergelijking met patiënten met een gemiddelde en hoge onderste slokdarm sfincter rustdruk. TLESRs zijn de belangrijkste mechanismen van reflux in gezonde vrijwilligers (82%). In patiënte met refluxziekte is het mechanismen waarop reflux plaats vindt afhankelijk van de LES rustdruk. In patiënten met refluxziekte die een hoge onderste slokdarm sfincter rustdruk hebben is het belangrijkste mechanisme van reflux: TLESR (72%). In patiënten met een lage onderste slokdarm sfincter rustdruk treedt gastro-oesofagiale reflux ook op tijdens TLESR (34%). Maar het belangrijkste gedeelte van de reflux episodes vindt plaats tijdens een lage/afwezige rustdruk. Wij hebben daarmee geconcludeerd dat: in patiënte met refluxziekte de frequentie van transiente relaxaties onder nuchtere condities niet significant verschilt van de controle groep, en dat na de maaltijd de frequentie zelfs significant lager ligt.
Ten tweede zijn in patiënten met refluxziekte significant meer TLESR geassocieerd met reflux. Ten derde blijkt dat in patiënten met refluxziekte TLESRs de belangrijkste

mechanismen van reflux zijn waarbij twee subtypen van refluxers te onderscheiden zijn (TLESR dominant versus incompetente onderste slokdarmsfincter).
En ten vierde, de onderste slokdarm sfincterdruk en niet de hernia diafragmatica of de endoscopische graad van oesofagitis bepaald het reflux mechanisme.

Distensie van de proximale maag is een sterke stimulus voor TLESR. In *hoofdstuk 3* hebben we de aanwezigheid van TLESRs, "common cavities" (als indicator voor gastro-oesofageale reflux van gas) en het opboeren van lucht bestudeerd na een gestandaardiseerde acute rek van de proximale maag, met behulp van lucht insufflatie in een controle groep, patiënten met refluxziekte en patiënten na anti-refluxchirurgie. Distensie van de proximale maag kan TLESRs provoceren. Maar patiënte met refluxziekte (3.1 TLESR/20 min) verschillen niet van de controle groep (3.5 TLESR/20 min). In patiënten na anti-reflux chirurgie is de frequentie van TLESR (1.8 TLESR/20 min) significant lager. "Common cavities" trof men met name aan na relaties van onderste slokdarm sfincter. Het belangrijkste mechanisme waardoor common cavities ontstaan zijn TLESR (50-60%). Na anti-reflux chirurgie blijkt dat intergastische insufflatie van lucht een verstoorde capaciteit tot opboeren van lucht laat zien in twee-derde van de patiënten terwijl ongeveer de helft van hen symptomen heeft in het dagelijkse leven.

De barostat techniek is gebruikt om continue distenties van de proximale maag te bestuderen (*hoofdstuk 4)*. Distensie van de proximale maag verhoogt de frequentie van transiente relaxaties (2.1-3.5 TLESR/15min). Er ontstaat echter een adaptatie na 15 minuten. In de tweede periode van 15 minuten is de frequentie te vergelijken met de basale periode. De frequentie van TLESRs gedurende de distensie van de proximale maag is gerelateerd aan de druk en de "wall tension" en niet zozeer aan het volume van de intergastrische balon.

In *hoofdstuk 5* laten we zien dat gastrine-17 de onderste slokdarm sfincter rustdruk doet verlagen wanneer het geïnfundeerd wordt in fysiologische hoeveelheden zoals gezien is bij postprandiale plasmaspiegels. Gastrine-17 had geen invloed op TLESRs alhoewel gastrine de gastro-oesofageale zure reflux deed toenemen (20 versus 55 reflux episodes). Tevens was het percentage van TLESRs geassocieerd met reflux toegenomen (20 versus 61%).

In *hoofdstuk 6* wordt beschreven dat somatostatine in de gezonde vrijwilligers, onder nuchtere omstandigheden, geen effect heeft op de onderste slokdarm sfincter rustdruk, nog op de TLESRs frequentie (2.1 versus 3.3 TLESR/uur). Gedurende een somatostatine infuus trad er geen daling van de onderste slokdarm sfincter rustdruk op zoals er normaal gesproken wel wordt gezien maar de onderste slokdarm sfincter druk nam langzaam toe. Na de maaltijd nam de frequentie TLESRs toe (2.6 versus 4.3 TLESR/uur). Somastostatine voorkwam echter niet alleen de postprandiale daling van onderste slokdarm sfincter rustdruk, maar ook de postprandiale toename van de TLESR frequentie.

Bombesine ofwel gastrine releasing peptide is bekend voor het feit dat het de onderste slokdarm sfincter rustdruk doet toenemen in gezonde vrijwilligers. In *hoofdstuk 7* hebben we het effect bestudeerd van bombesine op de onderste slokdarm sfincter rustdruk en op het effect TLESR. Bij patiënten met refluxziekte en patiënten na anti-refluxchirurgie gaf bombesine een significante toename van de onderste slokdarm sfincter rustdruk. Een

belangrijke bevinding was dat gedurende bombesine gestimuleerde condities van de onderste slokdarm sfincter complete sfincter relaxaties (TLESRs) nog steeds konden plaats vinden. De frequentie van TLESRs werd niet beïnvloed door bombesine niet onder nuchtere condities en nog na distensie van de proximale maag met lucht.

Wij hebben het effect van intraveneus L-arginine bestudeerd in een NO-donor op de karakteristieken van de onderste slokdarm sfincter in *hoofdstuk 8*. Inname van een koolhydraat-, vetrijke maaltijd doet de frequentie van TLESRs significant toenemen (2.7 tot 4.5 TLESR/uur). Intraveneuze L-arginine toediening resulteert in een toename van de plasmaspiegels van L-citrulline welke een indicator is voor een toegenomen NO- productie. L-arginine had geen significante invloed op de onderste slokdarm sfincter rustdruk, de TLESR of motiliteit van de tubulaire slokdarm zowel onder nuchtere als postprandiale condities.

In *hoofdstuk 9* is de sphinctometer vergeleken met de water-geperfundeerde sleeve. Onderste slokdarm sfincter rustdruk en sfincter relaxaties gemeten door de sleeve en sphinctometer vertoonden identieke patronen. Wel blijkt dat de sphinctometer output significant lager is dan de output van de sleeve. In gezonde vrijwilligers met een normale onderste slokdarm sfincter rustdruk registreert de sphinctometer TLESRs met vergelijkbare resultaten in vergelijking met de sleeve. Echter wanneer de onderste slokdarm sfincter rustdruk laag is blijkt dat het werkelijke aantal TLESRs wordt onderschat door de sphinctometer. Wanneer de criteria voor sphinctometer worden aangepast resulteerd dit in een verbetering van de herkenning van TLESRs. Met deze nieuwe criteria blijkt dat meer dan 90% van de TLESRs die met de sleeve worden herkend ook kunnen worden geclassificeerd met de sphinctometer. Aan de andere kant werden TLESRs ook gemist door de sleeve registratie terwijl ze duidelijk zichtbaar waren op de sphinctometer-registratie. Daarom lijkt het dat de sleeve niet langer aangehouden kan worden als de gouden standaard voor TLESR registratie.

In *hoofdstuk 10* bestuderen we de karakteristieken van de onderste slokdarm sfincter in gezonde vrijwilligers en patiënten met refluxziekte gedurende ambulante registratie met de sphinctometer. We laten zien dat er een dag-, nachtritme van de onderste slokdarm sfincter rustdruk bestaat met verhoogde rustdruk gedurende de liggende ofwel slaapperiode. De frequentie van TLESRs is toegenomen na de maaltijd en afgenomen gedurende de periode in liggende positie. In gezonde vrijwilligers en patiënten met refluxziekte werden TLESRs herkend als de belangrijkste mechanismen van de reflux (71 versus 51 %). Wij konden concluderen dat de sphinctometer als lichtgewicht manometrie registratie apparaat meer aandacht verdient en een rol kan spelen in de evaluatie van onderste slokdarm sfincter karakteristieken met name onder ambulante omstandigheden. Hoewel wel aangemerkt dient te worden dat de sphinctometer niet het ideale instrument is om absolute onderste slokdarm sfincter drukken te meten.

In *hoofdstuk 11* zijn in patiënten met refluxziekte en in gezonde vrijwilligers de mechanismen van peristaltische klaring van zure reflux door de tubulaire slokdarm bestudeerd. Tijdens een reflux episode is de frequentie van peristaltische contracties en contractiegolven significant verlaagd in patiënten met ernstige zure reflux. Het aantal contracties dat benodigd is om de tubulaire slokdarm te klaren van haar zure inhoud was bijna gelijk in alle vier subgroepen

(range 3.0-3.6 contracties per reflux episode). De karakteristieken van de tubulaire slokdarmcontracties, zoals amplitude en duur van de contracties, zijn niet significant verschillend in patiënten met ernstige zure reflux in vergelijking met patiënten met reflux in de normale hoeveelheid.

In *hoofdstuk 12* hebben we effect van laparoscopische Nissen fundoplicatie bestudeerd op de reflux mechanisme in een prospectieve studie. De laparoscopische Nissen fundoplicatie verhoogt significant de onderste slokdarm sfincter rustdruk (13-22 mmHg) en reduceert significant de frequentie van TLESRs (nuchter: 2.5 tot 0.6 TLESR/uur; postprandiaal: 4.0 tot 1.3 TLESR/uur). De laparoscopische Nissen fundoplicatie vermindert significant de blootstelling van de slokdarm aan zuur en reduceert de mechanismen van reflux. Echter de belangrijkste karakteristieken van de onderste slokdarm sfincter blijven na de maaltijd bestaan.

Tabel 1. Factoren van invloed op de karakteristieken van de onderste slokdarm sfincter.
≡ *Er is geen significant effect;* ↑ *toename;* ↓ *afname*

	TLESR frequentie	Onderste slokdarm sfincter rustdruk
Maaltijd	↑	↓
Distensie van de maag	↑	(↓)
Fundoplicatie	↓	↑
Gastrine	≡	↓
Bombesine	≡	↑
Somatostatine	≡	≡
Somatostatine + maaltijd	≡	↑
L-arginine (NO-precursor)	≡	≡
L-arginine + maaltijd	≡	≡

DISCUSSIE

Het doel van dit proefschrift was om de rol van de TLESR te bestuderen in gastro-oesofageale refluxziekte.

Inname van een maaltijd doet de frequentie van TLESRs toenemen. Distensie van de proximale maag speelt een belangrijke rol in deze postprandiale toename van de frequentie van TLESR, omdat distensie van het proximale maag met lucht en distensie met een intergastrische ballon beide een significante toename geven van het aantal TLESRs. Gastro-intestinale peptiden en neurotransmitters zoals gastrine, somatostatine, bombesine en en L-arginine hebben geen invloed op de frequentie van TLESRs. Echter deze peptiden konden wel de onderste slokdarm sfincter rustdruk beïnvloeden. Intraveneuze toediening van gastrine verlaagde de sfincterdruk bij postprandiale plasmaspiegels. Somatostatine onderdrukte de postprandiale afname van onderste slokdarm sfincter rustdruk en bombesine geeft een toename van onderste slokdarm sfincter rustdruk. Een belangrijke bevinding was dat gedurende een bombesine gestimuleerde verhoogde onderste slokdarm sfincter drukken TLESR nog steeds kunnen plaats vinden. Deze vindingen suggereren dat TLESRs onafhankelijk van onderste slokdarm sfincter rustdruk ontstaan.

In de gezonde vrijwilligers blijkt dat TLESRs de belangrijkste mechanismen van reflux zijn zoals dat ook het geval is in patiënten met refluxziekte met een normale of hoog normale onderste slokdarm sfincter rustdruk. Maar bij patiënten met een lage rustdruk is de frequentie van TLESR significant lager en zure reflux blijkt in deze patiënten met name door andere mechanismen dan TLESRs op te treden. Deze bevindingen suggereren dat de mechanismen van de reflux afhankelijk zijn van de onderste slokdarm sfincter rustdruk. Daarom is de onderste slokdarm sfincter rustdruk een belangrijke indicator voor het refluxtype en de reflux mechanismen.Verschillende typen refluxers lijken te bestaan, patiënten met reflux gedurende TLESR en patiënten met een incompetente gastro-oesofageale overgang.

Het middel van keuze in de behandeling van gastro-oesofageale relfux is een maagzuurremmend geneesmiddel zoals de protonpompremmer. De zure maaginhoud is hierbij in belangrijke mate beïnvloed. Echter de slokdarm motiliteit is niet beïnvloed. TLESRs zijn door anti-reflux chirurgie (laparoscopische Nissen fundoplicatie) effectief onderdrukt. Anti-reflux chirurgie vermindert de blootstelling van zuur aan de slokdarm en verminderd het aantal symptomen van refluxziekten. Het optreden van TLESR na anti-refluxchirurgie is belangrijk voor het behoud van de mogelijkheid tot het opboeren van intergastrische opgehoopte lucht. Ten tweede zou de onmogelijkheid tot complete relaxatie van de onderste slokdarm sfincter kunnen resulteren in dysfagie. We hebben patiënten met reflux bestudeerd voor en na anti-refluxchirurgie. Het is belangrijk om de motiliteit van de tubulaire slokdarm te meten voor een chirurgische behandeling. Met het gebruik maken van slokdarm manometrie met name door het meten van de slokdarm rustdruk is het mogelijk om verschillende types refluxers te onderscheiden. Maatwerk kan op deze manier aan patiënten met refluxziekten worden gegeven. Enerzijds voor medicamenteuze therapie, anderzijds chirurgische of endoscopische interventies. Momenteel worden endoscopische anti-reflux procedures vaker uitgevoerd. De eerste rapportages laten zien dat reflux symptomen afnemen en dat er minder afhankelijkheid is van een protonpompremmer. Het effect van de endoscopische anti-refluxprocedure op de onderste slokdarm sfincter karakteristieken zoals

TLESRs en sfincter rustdruk moet worden geëvalueerd en vergeleken worden met de resultaten van de laparoscopische anti-refluxchirurgie.

Ambulante meting van de motiliteit van de tubulaire slokdarm laat alleen maar geringe verschillen zien in amplitude en duur van de peristaltische slokdarmcontracties bij patiënten met ernstige relfuxziekten zien. Gedetailleerde informatie tijdens een zure reflux episode toont een afname van de frequentie van peristaltische contracties en contractiegolven in patiënten met ernstige reflux om de slokdarm te klaren van zure inhoud. Met het gebruik maken van de sphinctometer tijdens ambulante registratie is er een dag-, nachtritme te zien van zowel de TLESR en onderste slokdarm sfincter rustdruk. Gedurende een liggende nachtelijke periode is de TLESR frequentie lager in vergelijking met de periode overdag. Het meten van de slokdarm en de onderste slokdarm sfincter motiliteit is behulpzaam in het bestuderen van molitiliteitsstoornissen en de gevolgen van interventies zoals chirurgische of endoscopische antireflux procedures.

In conclusie: we hebben de motiliteit van de slokdarm bestudeerd met name TLESR en onderste slokdarm sfincter rustdruk in gezonde controles, patiënten met refluxziekte en patiënten na laparoscopische Nissen fundoplicatie. De onderzoeken hebben geresulteerd in een betere kennis over de functie van de slokdarm, de gastro-oesofageale overgang als barrière voor gastro-oesofageale reflux. TLESR zijn spontane relaxaties van de onderste slokdarm sfincter welke kunnen worden gestimuleerd door distensie van de proximale maag. TLESR kunnen worden onderdrukt, maar afwezigheid of ernstige onderdrukking van deze complete relaxaties kunnen de veiligheidsklep-functie van de onderste slokdarm sfincter negatief beïnvloeden. Dit is het opboeren van overtollige intergastrische lucht.

CURRICULUM VITAE

Jan Willem Straathof werd geboren op 14 juli 1965 te Eindhoven. In 's-Gravenhage vervolgde hij het Gymnasium beta aan het Aloysius College, alwaar in 1984 het V.W.O. diploma werd gehaald. Hij begon zijn medische opleiding aan de Rijks Universiteit van Antwerpen in 1984. Na een jaar werd de studie geneeskunde vervolgd aan de Universiteit Leiden, alwaar hij in 1992 het doctoraal examen en in het begin van 1994 het artsexamen aflegde. Tijdens deze studie begon hij met wetenschappelijk onderzoek als student-assistent op het Laboratorium voor Experimentele Chirurgie van het Academisch Ziekenhuis Leiden met dierexperimenteel onderzoek van pancreastransplantatie. In 1991 reisde hij naar Adelaide in Australië om daar met Michael Horowitz en John Dent onderzoek te doen op het gebied van motiliteit van de slokdarm en de maag. Na het artsexamen voldeed hij zijn militaire dienstplicht als marine arts aan boord van Hr.Ms. Van Amstel. Van 1995 tot 1999 werkte hij als klinisch onderzoeker op de afdeling Maag-, darm- en leverziekten van het Leids Universitair Medisch Centrum. Samen met Ad Masclee deed hij onderzoek op het gebied van motiliteit van de slokdarm en de maag met name naar transiente onderste slokdarm sphincter relaxaties. De hieruit voortvloeiende onderzoeks resultaten zijn beschreven in deze thesis.
In maart 1999 startte hij met de vooropleiding Interne Geneeskunde in het Rode Kruis Ziekenhuis te 's-Gravenhage (opleider: Dr. R.M. Valentijn). De opleiding tot Maag-, darm- en leverarts werd in 2002 voortgezet in het Leids Universitair Medisch Centrum (opleider: prof. dr. C.B.H.W. Lamers). Sinds 1 mei 2005 is hij geregistreerd als maag-, darm- en leverarts en is alsdanig werkzaam in het Máxima Medisch Centrum te Veldhoven/Eindhoven. De auteur is gehuwd met Mariëtte Oostindiër. Zij hebben vier kinderen: Sabine, Dirk Jan, Elianne en Arnout.

NAWOORD

Wetenschappelijk onderzoek doe je niet alleen. Uiteindelijk heeft een heel team in meer of mindere mate bijgedragen aan de onderzoeken en later de tot stand koming van dit proefschrift.

Het begon allemaal aan de andere kant van de wereld in Australië, waar ik wat meer wetenschappelijk onderzoeks ervaring wilde opdoen. In een bijzondere omgeving zaten enthousiaste mensen die diepgravend en vernieuwend onderzoek deden naar motiliteit van slokdarm en maag. Terug in Leiden bleek er eenzelfde enthousiasme te zijn op de afdeling maag-, darm- en leverziekten van het Academisch Ziekenhuis. De eerste studie met gastrine was succesvol en de pilot met luchtinsufflatie en bombesine gaf hoopvolle resultaten. Het wordt dan moeilijk om een baan te weigeren. Zeker wanneer je op zee per spoedbericht hiervoor gevraagd wordt.
Vele studenten hielpen met metingen en konden hierover een afstudeerverslag schrijven: Marieke Adamse, Margreet Lüchtenborg, Renske van der Meer, Steven Tieleman en Maaike van Veen zijn daarom ook medeauteur van verschillende publicaties.
Omdat luchtinsufflatie in de maag zijn beperkingen had, was de barostat eigenlijk de aangewezen techniek om beter onderzoek te doen naar de maag en slokdarm. Dit resulteerde in een tweede onderzoekslijn met extra onderzoeken samen met Banafsche Mearadji, waarop zij inmiddels is gepromoveerd.
De computer is onmisbaar in de verwerking van alle data. Oude programmatuur, zoals HG3, Dbase, en Polygram blijven onvolprezen goed functioneren ondanks de intrede van Windows. Een speciaal woord voor Hugo Gielkens, eertijds medeonderzoeker. Zonder zijn kleine computerprogramma's waren statistisch berekeningen zoals MANOVA ondoenlijk. Het onderzoek naar peristaltiek van de slokdarm met 24 uurs registratie was mogelijk omdat we de data uit de registratie-software wisten te verzamelen. Dat de functieafdeling hiervan profiteerde met geautomatiseerde verslaglegging was mooi meegenomen.
Ondersteuning in welke vorm dan ook kreeg ik van de verpleegkundigen van de functie- en endoscopieafdeling Carlien de Jong, Atie Jongma, Cindy Magdalena en Betty van Velzen. Technische support van Ruud Kamerling. Secretariële ondersteuning van Jolet Kerkvliet, Jenneke Hofkes, Maritza Koster-de Vreese en Loes Niepoth. Laboratorium ondersteuning van Wim van Duin, Eveline Muller, Jan Paul Gilliams (†), Hein Verspaget en Izak Biemond.
Morele support kwam er van collega's maar vooral van vrienden en familie. Al is het soms moeilijk uit te leggen hoe lang de opleiding van een arts is!
Hoe moet je trouwens mensen bedanken die er niet meer zijn? *Memento Mori!*
In de tien jaar die zijn verstreken heb ik niet stilgezeten. Het gezin groeide uit naar vier kinderen. Onderzoeksresultaten werden gepresenteerd in Veldhoven, Aken, Parijs, Birmingham, San Francisco, Washington en New Orleans. Na het onderzoek volgde de opleiding tot maag-darm-leverarts, waarbij het perspectief van de reflux ziekte binnen de maag-darm-leverziekten werd verlegd naar de klinische en endoscopische beoordeling. Transiente onderste slokdarm sfincter relaxaties ofwel TLESR hebben nauwelijks bekendheid als oorzaak van opboeren en reflux. Hopelijk draagt dit proefschrift bij aan de promotie van een fysiologisch fenomeen.
In al die jaren is er maar een persoon, die het belangrijkste klankbord was en is:
Mariëtte Oostindiër. Dank je :-)