CHAPTER 1

Gastroesophageal Reflux Disease: Pathophysiology

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Key points

- The anti-reflux barrier does not solely consist of the intrinsic pressure generated by the lower esophageal sphincter, but is complemented by the extrinsic pressure exerted by the crural diaphragm and the presence of the flap valve.
- Transient lower esophageal sphincter relaxations constitute the main mechanism of reflux in gastroesophageal reflux disease patients and healthy subjects.
- The presence of a hiatal hernia increases the severity of esophageal acid exposure, and changes the position of the acid pocket.
- The severity of gastroesophageal reflux disease-related symptoms is not predicted by the severity of esophageal acid exposure and is dependent on factors influencing the perception of reflux.
- Dilated intercellular spaces are more frequently present in non-erosive reflux disease patients and possibly contribute to symptom generation.

Introduction

Over the past decades, considerable changes in our understanding of gastroesophageal reflux disease (GERD) have taken place. In the era before widespread application of endoscopy, when radiography was the only diagnostic tool available, the diagnosis of GERD was more or less synonymous with hiatal hernia. After the introduction of flexible esophagogastroduodenoscopy, mucosal lesions in the distal esophagus became the most important characteristic of the disease. Nowadays, we know that reflux symptoms can be present in the absence of reflux esophagitis. This subset of the disease is labeled non-erosive reflux disease (NERD). In addition, extraesophageal symptoms and signs, such

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as laryngitis, gastric asthma and chronic cough, were recognized. The Montreal definition encompasses all of these elements of the disease by stating that it is characterized by either bothersome symptoms and/or lesions caused by reflux of gastric contents. This gradual broadening of our understanding of what GERD is has led to an expansion of our concepts of the pathophysiology of the disease [1]. Whilst the factors that determine the exposure of the esophageal mucosa to gastric contents are still relevant to the pathophysiology of GERD, factors that affect the sensitivity of the esophagus have become recognized as equally important. This chapter aims to summarize the many factors that are presently seen as important in the pathophysiology of GERD.

Mechanisms leading to gastroesophageal reflux

Anti-reflux barrier

In the early days after the advent of esophageal manometry, the lower esophageal sphincter (LES) was conceptually prominent in the pathophysiology of GERD. A LES able to maintain a sufficiently high pressure at the esophagogastric junction (EGJ) was considered to be the most important factor preventing gastroesophageal reflux. Nowadays, the anti-reflux barrier is thought to consist of intrinsic LES pressure, extrinsic compression of the LES by the crural diaphragm, and the "flap valve" constituted by an acute angle of His.

Lower esophageal sphincter

The LES is a 3–4 cm segment of tonically contracted smooth muscle at the EGJ. Normally, the LES is surrounded by the crural diaphragm. When a sliding hiatus hernia is present, the LES is proximal to the crural diaphragm (Figure 1.1). Resting LES tone, best measured during end-expiration, varies among normal individuals from 10 to 30 mmHg relative to intragastric pressure. Within a subject, LES pressure varies considerably during the day. The highest pressure occurs during phase III of the migrating motor complex, during which it may exceed 80 mmHg. Immediately after a meal, LES pressure typically decreases. The genesis of LES tone is a property of both the smooth muscle itself and of its extrinsic innervation.

Lower esophageal sphincter pressure is affected by myogenic factors, intraabdominal pressure, gastric distension, peptides, hormones, various foods, and many medications.

Crural diaphragm

The opening in the diaphragm through which the esophagus reaches the abdomen (hiatus esophagei) is shaped like a teardrop. In the absence of a

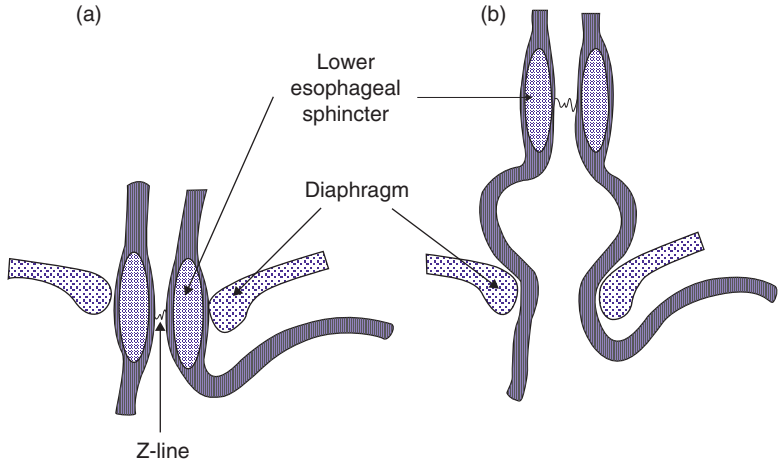


Figure 1.1 Position of the LES with respect to the crural diaphragm. (a) Normal morphology. (b) Hiatus hernia.

hiatal hernia, the LES is surrounded at this point by the crural diaphragm, i.e. the right diaphragmatic crus. Especially during inspiration, the crural diaphragm contributes to the maintenance of EGJ competence. For this reason, the crural diaphragm is often referred to as the extrinsic sphincter, the smooth muscle of the LES being the intrinsic sphincter. This situation resembles that of the internal and external sphincters surrounding the anal canal.

Flap valve

A third component of the anti-reflux barrier at the EGJ is constituted by the so-called flap valve, formed by a musculomucosal fold created by the entry of the esophagus into the stomach along the lesser curvature. With this anatomical arrangement, increased intraabdominal or intragastric pressure compresses the subdiaphragmatic portion of the esophagus. This is supposed to prevent EGJ opening and reflux during periods of abdominal straining. Hill and colleagues proposed a grading scheme based on the endoscopic appearance of the gastroesophageal flap valve during retroflexion (Plate 1.1).

Mechanisms of reflux

Current thinking is that there are three dominant reflux mechanisms: transient LES relaxations, LES hypotension, and anatomical distortion of the EGJ, e.g. hiatus hernia. Transient LES relaxations (TLESRs), constituting the most important reflux mechanism in healthy subjects and in a large subset of GERD patients, will be discussed in greater detail in the next paragraph.

When diminished LES pressure is present (either with or without anatomical abnormality), short-lived increases in intraabdominal pressure caused by straining are often the precipitating factor of the reflux. Manometric data suggest that this rarely occurs when the LES pressure is greater than 10 mmHg [2]. It is also a rare occurrence in patients without hiatus hernia [3]. Free reflux is characterized by a fall in intraesophageal pH without an identifiable change in either intragastric pressure or LES pressure. Episodes of free reflux are observed only when the LES pressure is lower than 5 mmHg.

It is important to realize that EGJ relaxation as measured manometrically does not equate to EGJ opening or EGJ compliance, which are likely to be more relevant to the occurrence of reflux. EGJ compliance can be assessed with a water-filled balloon straddling the EGJ and measurement of the diameter of the balloon at various levels of filling. In patients with hiatus hernia, the compliance of the EGJ is increased but even patients without hiatus hernia may have increased EGJ compliance. In the latter, defects not readily detectable with imaging techniques, such as an abnormal gastroesophageal flap valve, defects in the LES musculature or a wide diaphragmatic hiatus, are thought to be present. Subtle differences in EGJ opening and compliance are likely to explain the discriminatory function of the EGJ: large volumes of gas can be vented from the stomach while at the same time fluid is largely contained within the stomach.

Transient lower esophageal sphincter relaxations

Function and definition

Lower esophageal sphincter relaxations are common and occur mainly during swallows to allow passage of a bolus into the stomach [4]. In addition, the LES can relax during the so-called TLESR which occurs less frequently, about 3–6 times per hour [5,6]. TLESRs are considered the physiological mechanism which enables venting of gas from the stomach, also known as belching [7]. This belching reflex acts as a protective mechanism which prevents excess amounts of gas accumulating in the stomach. Since the discovery of TLESRs in the early 1980s, it has become increasingly clear that most reflux episodes occur during TLESRs [8]. Other mechanisms which can induce reflux episodes include straining, coughing, and free reflux. However, these mechanisms only become important – relatively and absolutely – in patients with severe reflux disease associated with hiatal hernia.

A TLESR is currently defined as an abrupt decline in pressure at the position of the LES which is not induced by swallowing [9]. Additional criteria which could be helpful but are not needed for the identification of TLESRs are crural diaphragm inhibition and a prominent after-contraction [10]. Since the definition of TLESR is based solely on the

esophageal pressure profile, the gold standard by which to measure TLESRs is esophageal manometry (Plate 1.2).

Pharyngeal stimulation can also result in an LES relaxation which resembles a TLESR [4]. However, LES relaxations induced by pharyngeal stimulation are rarely associated with inhibition of the crural diaphragm and acid reflux [6]. Furthermore, esophageal reflux was found only when an LES relaxation was associated with diaphragm inhibition [6].

Mechanisms of transient lower esophageal sphincter relaxation

The primary stimulus which triggers a TLESR is gastric distension, often resulting from accumulation of gastric air or consumption of a meal. Distension in any part of the stomach can trigger a TLESR. However, the subcardiac region of the stomach showed the lowest threshold for triggering TLESRs [11]. While still under debate, several studies suggest that tension receptors in the stomach appear to be more relevant than pressure receptors as the stimulus for transient LES relaxation [12,13].

Transient LES relaxations are characterized by four different events. The concerted action of these events results in complete relaxation of the EGJ. The first and most prominent event during a TLESR is relaxation of the inner part of the LES [14]. The second event is relaxation of the crural diaphragm [15]. The third event is suppression of esophageal peristalsis [14] and the fourth is a contraction of the distal esophageal longitudinal muscle leading to esophageal shortening [16]. It has been hypothesized that the longitudinal muscle contraction of the distal esophagus may be the primary motor event leading to LES relaxation [17] but this hypothesis remains to be proven.

Relaxation of the EGJ during a TLESR is terminated by primary peristalsis or, more commonly, by secondary peristalsis [18]. Swallow-induced primary peristalsis is characterized by upper esophageal sphincter (UES) relaxation with pharyngeal contraction and esophageal peristalsis progressing along the entire esophagus. Secondary peristalsis is defined as a wave in the esophagus which is not associated with UES relaxation and is a result of esophageal distension, often arising from gastroesophageal reflux.

The rate of TLESRs can vary greatly during the day. The postprandial period is characterized by a four- to fivefold increase in the rate of TLESRs and an increase in the proportion of TLESRs accompanied by reflux [19]. Body position can also influence the rate of TLESRs since the incidence of TLESRs, as well as the incidence of reflux-associated TLESRs, is higher in the right recumbent position compared to the left recumbent position [20]. Furthermore, the rate of TLESRs is greatly decreased during the night [18]. This is in accordance with the observation that reflux episodes occur less often during the night than during the day [8]. Despite this nocturnal

decrease in the rate of TLESRs, a subset of GERD patients still shows substantial acid exposure during the night. Therefore, in patients with pathological nocturnal reflux, additional mechanisms are involved, such as free reflux through a mechanically incompetent sphincter [21].

The reflex pathway of the TLESR is a vagovagal reflex which commences with activation of gastric receptors primarily in the subcardiac region [11]. Sensory signals from the stomach are projected to the brain through afferent sensory fibers of the vagus [22] and its terminating synapses are located in the nucleus tractus solitarius (NTS) [23]. Signals from the NTS do not provide signals to the EGJ directly but are relayed to the caudal part of the dorsal motor nucleus of the vagus [24,25]. This central pathway which modulates TLESRs is shared by both the LES and crural diaphragm [26]. Furthermore, the crural diaphragm is innervated not only through efferent vagal endings but also by the phrenic nerve [27]. The brainstem sites responsible for this dual innervation are yet to be defined. Efferent motor function signals from the brain to the LES and crural diaphragm are conducted through the motor tract of the vagus [28]. Finally, motor signals are relayed through the myenteric plexus from where they are further distributed to the esophageal body and LES [28].

Many excitory and inhibitory neurotransmitters and receptors, including nitric oxide, opioids, anticholinergic agents and the neuropeptide CCK, have been found to play a role in the neuromodulation of TLESRs [29]. Among these neurotransmitters, the gamma-aminobutyric acid (GABA) and metabotropic glutamate receptors (mGluR), and the cannabinoid receptor 1 (CBR1) are of particular interest as potential targets for therapeutic interventions. The most extensively investigated neurotransmitter in the TLESR pathway is GABA-B. GABA-B acts as an inhibitory neurotransmitter, and its receptors are located at both central and peripheral sites in the TLESR reflex arch [30,31]. Metabotropic glutamate receptors are also present throughout the central and peripheral nervous system. The most extensively investigated metabotropic glutamate receptor is mGluR5 which has an excitatory function, mainly with a periperal site of action [32, 33]. The CBR1 has only recently been investigated with regard to TLESRs. Its site of action is believed to be the central nervous system [34].

Despite the importance of the TLESR in the pathophysiology of GERD, most of our knowledge regarding the neural pathways involved in the reflex arc of the TLESRs is derived from animal studies. However, it is assumed that TLESRs in humans follow similar pathways.

Association between gastroesophageal reflux and transient lower esophageal sphincter relaxations

Transient LES relaxations are considered to be the main mechanism leading to gastroesophageal reflux in GERD patients. However, the majority

of the studies show a similar rate of TLESRs in healthy subjects and GERD patients [35,36]. This means that in GERD patients, there is a higher percentage of TLESRs which not only vent air but are also associated with gastroesophageal reflux. Therefore, a different underlying mechanism is necessary which results in this loss of discrimination between air and liquid by the LES.

In GERD patients, a slightly higher transsphincteric pressure gradient is present before and during a TLESR when compared to healthy subjects [37]. More importantly, the pressure gradient is greater during TLESRs accompanied by acid reflux compared to TLESRs without acid reflux. Another proposed contributing factor is EGJ compliance, also known as EGJ distensibility. GERD patients are characterized by an increase in EGJ compliance which could explain the loss of discrimination between air and liquids [38]. Furthermore, EGJ compliance in GERD patients with hiatal hernia is increased compared to GERD patients without hiatal hernia [39]. Obesity is associated with an increased rate of TLESRs as well as with an increased association of TLESRs with gastroesophageal reflux [40]. In addition, a higher pressure gradient has been measured during TLESRs in obese subjects compared to normal-weight subjects. The influence of different nutritional factors on the association of TLESRs and reflux as well as the rate of TLESRs has been extensively studied. However, no correlation between reflux-associated TLESRs or an influence on the rate of TLESRs has been demonstrated.

Hiatal hernia

In 1971, Cohen and Harris published a paper in which they reported that reflux symptoms correlated with low LES pressure, rather than with presence of a hiatus hernia [41]. From then on, the emphasis in studies on GERD pathophysiology was on basal LES pressure. Another change took place when the phenomenon of TLESR was found to play a pivotal role [42]. The sleeve sensor that was required to record TLESRs did not allow recognition of the two distinct components of the high-pressure zone, i.e. LES and crural diaphragm. Awareness of the importance of hiatal hernia for the pathophysiology of GERD emerged again around the turn of the century. It is clear that esophageal acid exposure is greater in patients with hiatus hernia [3,43–45]. In addition, the severity of esophageal acid exposure increases with increasing size of the hernia [45,46] and esophagitis is more severe with more severe acid exposure [47]. Patients with Barrett's esophagus have the highest prevalence of hiatus hernia [48].

Hiatus hernia is not an all-or-nothing phenomenon. The so-called physiological hernia (also known as phrenic ampulla) is only present during swallowing when the esophageal shortening leads to displacement of the Z-line to a site proximal to the diaphragm. This displacement

is <2 cm. A reducing hiatal hernia is a hernia which is greater than 2 cm but which is only seen during a swallow; between swallows, the Z-line is at the level of the diaphragm. A non-reducing hiatal hernia is defined as a hernia greater than 2 cm in which the Z-line does not return to its normal position between swallows. At moments at which a hiatus hernia is present, the anti-reflux effect of the crural diaphragm is exerted at the wrong spot, i.e. distal to the LES, and the effect is weakened because the hiatus is usually wider than normal. Using pull-through manometry and three-dimensional representation of the pressure profiles, Kahrilas and co-workers demonstrated in hiatus hernia patients that there are distinct intrinsic sphincter and hiatal canal pressure components, with each one exerting pressure of lower magnitude than normal. Simulating reduction of the hernia by repositioning the intrinsic sphincter back within the hiatal canal and arithmetically summing superimposed pressures resulted in calculated EGJ pressures which were practically indistinguishable from those of the control subjects [49]. Prolonged manometric studies have also made clear that mechanisms other than TLESR play a more prominent role when a hiatus hernia is present. These other mechanisms include low LES pressure, straining-induced reflux and swallow-associated reflux [3].

Even within the same patient, the mechanisms leading to reflux vary from time to time, depending on the reduced or non-reduced status of the hiatus hernia [50]. Another mechanism by which the presence of a hiatus hernia is associated with excessive esophageal acid exposure is characterized by superimposed reflux from the hiatal sac during swallowing-induced LES relaxation. This can be seen in non-reducing hiatus hernias [51, 52].

Gastric factors

Total gastric emptying

It is tempting to speculate that delayed gastric emptying is an important factor in the pathogenesis of GERD. However, the evidence for this hypothesis appears to be controversial.

Numerous studies have observed delayed gastric emptying in a proportion of GERD patients compared to healthy controls [53] and only a few studies reported no difference. However, no correlation between esophageal acid exposure time and delayed gastric emptying could be proven [54]. Furthermore, acceleration of gastric emptying by cisapride was not associated with a decrease in esophageal acid exposure or with the number of reflux events [55]. Studies investigating the association between gastroesophageal reflux and gastric emptying are limited by measuring acidic reflux episodes only. To our knowledge, no study has been published which assesses the influence of gastric emptying on weakly acidic reflux episodes.

Emptying of the proximal stomach

Over the last few decades, the role of the proximal stomach in the pathogenesis of GERD has gained much attention since TLESRs are triggered by distension of the proximal stomach and the refluxate is located in the proximal stomach as well. The motor response of the proximal stomach to a meal is characterized by a relaxation followed by a gradual recovery of gastric tone. It has been found that GERD patients are characterized by a delayed recovery of proximal gastric tone after a meal compared to healthy controls [56]. Furthermore, emptying from the proximal stomach, but not the distal stomach, was significantly delayed in GERD patients compared to healthy controls.

Slow proximal emptying shows a correlation with increased esophageal acid exposure time [57]. Furthermore, the number of acidic reflux episodes correlates with proximal gastric retention [58]. Thus, in contrast to gastric emptying of the whole stomach, delayed emptying of the proximal stomach appears to be a factor in the pathogenesis of GERD. In theory, delayed emptying of the proximal stomach could cause an altered position of the postprandial acid pocket (see below) and influence the association of TLESRs with reflux. However, this hypothesis remains to be proven.

Acid pocket

Until recently it was assumed that gastric acid secreted after a meal is instantly mixed with the ingested food into one homogeneous mixture. The buffering effect of many food constituents leads to a postprandial increase in gastric pH. However, Fletcher *et al*. observed that the pH in the body of the stomach was markedly higher (pH 4.7) than the pH of the esophageal refluxate (pH 1.6) [59]. In subsequent pull-through pH studies, they identified a pocket of unbuffered gastric acid which lies on top of a homogenized fatty meal. This so-called acid pocket extends from the cardia to the distal esophagus [59].

The position of the acid pocket in GERD patients differs from healthy controls, i.e. a supradiaphragmatic localization of the pocket was more frequent in patients with GERD, especially those with a large HH (Plate 1.3) [60]. Localization of the acid pocket strongly correlates with the occurrence of acid reflux. When the acid pocket is located above the diaphragm, 70–85% of all TLESRs are accompanied by acid reflux [60]. In contrast, when the acid pocket is located below the diaphragm, only 7–20% of TLESRs are accompanied by an acidic reflux episode. Even during reflux episodes which are caused by mechanisms other than TLESRs, the position of the acid pocket is still of major importance.

Effect of posture on reflux

Body position does not affect the acidity in the gastric cardia and corpus. However, the right recumbent position is associated with an increase in

acid exposure time in the distal esophagus compared to the left recumbent position [61]. This is due to an increase in reflux episodes, TLESRs and TLESRs associated with reflux [20]. The duration of reflux episodes is not affected by body position.

Obesity

Overall, the weight of the evidence suggests that obesity and GERD are related. When dissected to individual aspects of the disease, there are areas of controversy. For instance, the results of studies on esophageal acid exposure – as measured with 24-h pH monitoring – in obesity are not entirely unequivocal [40,62–72]. Recent data indicate that the proximal esophageal extent of the refluxate is higher in obese subjects [73]. It is likely that, in the obese, waist circumference is a more important determinant of excessive reflux [65,66].

There are relatively few studies on LES function in the obese. The limited data available suggest that basal LES pressures in the morbidly obese are similar to those of ideal body weight [74]. However, obesity is associated with an increased incidence of TLESRs, the association being present for increased Body Mass Index (BMI) as well as waist circumference [40].

Hiatal hernia is found more often in patients with obesity than in subjects with a normal BMI [75,76]. Increased intragastric pressure may promote the development of hiatus hernia by applying an axial pressure strain through the diaphragm [77].

Apart from promoting the development of hiatus hernia, the increased intragastric pressure found in the obese tends to promote reflux. Especially during inspiration, increased intragastric pressure and the gastroesophageal pressure gradient are correlated with increased BMI. The changes noted above are more strongly correlated with waist circumference.

In summary, obese subjects are more likely to have a high incidence of TLESRs, a hiatal hernia, increased intragastric pressure, and an increased gastroesophageal pressure gradient. These factors all facilitate reflux. A positive association between reflux symptoms and BMI was found in more than a dozen studies. Two metaanalyses incorporating these studies confirmed the existence of such an association and found the risk of having reflux symptoms in the overweight and obese to be 43–94% higher than in normal-weight subjects [66,78]. In a study in women, a BMI > 30 kg/m^2 was associated with a threefold increase in the odds of having frequent reflux symptoms [79].

Despite the equivocal nature of the evidence for increased gastroesophageal reflux in the obese, a metaanalysis showed a statistically significant increase in the risk for esophageal lesions with increasing weight. A BMI greater than 25 kg/m^2 had an odds ratio of 1.76 for erosive esophagitis and 2.02 for esophageal adenocarcinoma, compared with patients with normal

weight [78]. Four prospective multicenter, randomized, double-blind trials comparing esomeprazole and other proton pump inhibitors found a weak but statistically significant increased risk for Los Angeles grades C and D esophagitis, but not grades A and B, in the obese [80]. In a case–control study that evaluated cases with Barrett's esophagus and two control groups (normal-weight patients and patients with GERD but without Barrett's esophagus), abdominal diameter was found to be an independent risk factor for Barrett's esophagus. There was no association between Barrett's esophagus and BMI [66].

Studies on the effect of weight loss obtained by non-surgical methods on reflux symptoms, endoscopic findings or pH monitoring have yielded somewhat disappointing results [81,82]. However, when studies describing surgically achieved weight loss are also taken into account, a positive conclusion can be drawn [83].

Mechanisms involved in perception of reflux

With the development of new techniques it has become clear that esophageal acid exposure is not the only factor involved in the generation of reflux symptoms, and that mechanisms altering the perception of gastroesophageal reflux must have an effect.

The addition of ambulatory pH measurement to the diagnostic armamentarium made it possible to not only quantify the severity of esophageal acid exposure, but also to assess the temporal relation between symptoms and acid reflux episodes. In order to describe this relationship between gastroesophageal reflux and symptoms, several tools have been developed. The one considered to have the fewest shortcomings is the Symptom Association Probability (SAP), proposed by Weusten *et al*. [84]. To calculate the SAP, the 24-h pH measurement is divided into 2-min time frames and the occurrence of reflux in these periods and in the 2-min time frame preceding the moments of symptom onset is noted. Thereafter the probability that symptoms are associated with reflux is calculated. The SAP is considered to be positive once it is >95%.

Using the SAP, it has become apparent that esophageal acid exposure is not closely related with the number of reflux symptoms experienced by the patient and that acid exposure and positive symptom-reflux associations are largely independent phenomena [85]. This is in contrast to the finding that as the severity of esophageal acid exposure increases, this is accompanied by an increasing severity of erosions [47]. When a patient's esophagus is exposed to physiological acid reflux and there is no correlation between symptoms and the reflux episodes (negative SAP), he or she is classified as having "functional heartburn." When physiological reflux is

present and bothersome reflux symptoms appear to be correlated with that reflux, the patient is considered to have a "hypersensitive esophagus." In patients with pathological esophageal acid reflux, the distribution between those with a positive and a negative SAP is not different from the distribution in patients with physiological esophageal acid exposure, suggesting that symptom generation is mostly independent of the severity of the reflux [85].

Intraluminal factors influencing perception and thereby symptom generation include several reflux characteristics. First, reflux episodes preceded by a higher cumulative acid exposure time are more likely to be perceived. The difference in cumulative acid exposure time between symptomatic and asymptomatic reflux episodes is apparent for up to 75 min [86]. Furthermore, symptomatic reflux episodes have a higher median proximal extent and a longer median duration [87]. However, it must be considered that there is an overlap in proximal extent between symptomatic and asymptomatic reflux episodes and therefore an individual threshold above which a reflux episode will always be symptomatic cannot be established.

Non-acid reflux

The introduction of combined pH and impedance monitoring broadened the spectrum of gastroesophageal reflux since the technique allows further characterization of reflux episodes according to acidity and composition (liquid or mixed liquid-gas). By the addition of impedance, reflux episodes without a pH drop that would have been missed with a conventional ambulatory pH measurement can be detected. Thereby the new phenomenon of non-acid reflux emerged. Whereas it was long felt to be unlikely that non-acid reflux can provoke symptoms, results of a perfusion study carried out two decades ago had indicated that non-acid solutions with pH up to 6 exacerbate symptoms in around 50% of subjects [88]. We now know that esophageal exposure to non-acid gastric content is a possible explanation for the persistence of symptoms after adequate acid-suppressive therapy.

Using impedance measurement, it has been shown that acid suppression with a proton pump inhibitor (PPI) reduces neither the total number of reflux events nor their proximal extent. Rather, PPI treatment decreases the number of acid reflux in favor of weakly acidic (nadir pH between 4 and 7) and alkaline (nadir pH > 7) reflux [89].

Non-acid reflux proved to be responsible for 15% of symptomatic reflux episodes in patients off PPI [86]. In patients on PPI therapy presenting with persistent reflux symptoms, 37% of subjects showed a positive Symptom Index (SI) for non-acid reflux. This emphasizes the possible role of impedance measurement in identifying this subgroup of patients who could

benefit from additional therapy aimed at reducing the absolute number of reflux events (TLESR inhibitors, fundoplication) [90]. The most interesting finding made with impedance monitoring is that the majority of patients with persisting symptoms under PPI therapy show a negative symptom index for acid and non-acid reflux, suggesting an erroneous initial diagnosis and supporting the possibility of stopping PPI therapy.

As mentioned, the composition of the refluxate differs, with about half of total reflux episodes being completely liquid and half having a gaseous component, which is similar in GERD patients and healthy volunteers. However, the reflux episodes causing symptoms in NERD patients more often contain a gaseous component [91].

Dilated intercellular spaces

The mechanical barrier that lies between luminal acid gastric content and esophageal nociceptors is the esophageal epithelium. The human esophageal epithelium is a stratified squamous epithelium consisting of three layers: the upper layer is the stratum corneum or so-called functional layer, below which lies the stratum spinosum or prickle cell layer. Finally, on the serosal side of the epithelium, the stratum basale is located. A functional epithelial barrier function is maintained by desmosomes and tight junctions. Desmosomes enable strong cell-to-cell adhesion by linking cell surface adhesion proteins to intracellular keratin cytoskeletons. They are present throughout the three layers of esophageal epithelium but are most frequently located in the prickle cell layer [92]. In addition, tight junctions seal the intercellular space and prevent the paracellular diffusion of fluid and small molecules.

Several histopathological changes in the esophageal epithelium of GERD patients have been described, such as thickening of the basal cell layer, elongation of mucosal papillae [93] and dilated intercellular spaces (DIS) [94]. Since Tobey *et al*. first described DIS in NERD patients [95], the phenomenon has been extensively studied and proposed as a possible key mediator of symptom generation in GERD patients. DIS can be seen as a dysfunction of the epithelial barrier function, enabling the diffusion of fluid and acid molecules into the intercellular space and allowing them to reach and activate chemosensitive nociceptors in the underlying layers [96].

Several studies have assessed DIS in human esophageal biopsy samples, some of which used transmission electron microscopy (TEM), allowing accurate measurement of the intercellular space (Figure 1.2) [95,97,98]. These studies found that the mean diameter of intercellular spaces in NERD patients (1.0–2.2 µm) is at least twice that in healthy controls (0.45–0.56 µm) [99]. This suggests that DIS measurement by TEM in biopsies is a useful tool to confirm the otherwise difficult diagnosis of NERD. However, TEM is expensive and time-consuming and therefore it does not seem

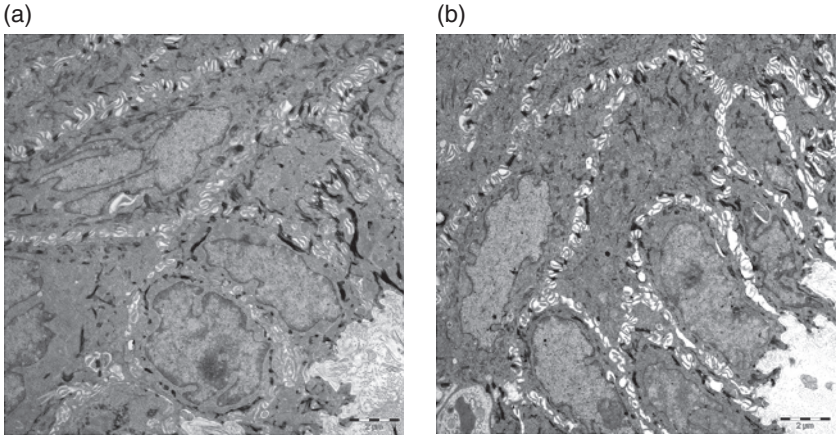


Figure 1.2 Transmission electron microscopy image of the basal layer of rat esophageal mucosa. (a) Normal morphology. (b) Dilated intercellular spaces in a rat treated with a moderate stressor.

easily applicable in clinical practice. Multiple studies have tried to measure intercellular space diameters using the more accessible technique of light microscopy (LM) [100,101]. However, the results regarding the variability between TEM and LM are conflicting and the correlation between measurements performed by the two techniques does not seem to be very promising [102,103].

The exact mechanism responsible for the generation of DIS has not been elucidated. Since exposure of esophageal mucosa to gastric contents was the first logical explanation, *in vitro* and *in vivo* studies have primarily focused on their relation with DIS.

Exposure of rabbit esophagus to an acidic solution with pH 1.1 causes no macroscopic erosions but shows clear DIS under TEM, which is accompanied by a drop in epithelial resistance and an increase of esophageal permeability to small molecules [104]. The addition of pepsin to an acidic solution further increased the rate of DIS, but the effect was only present with pH < 3 [105]. Besides acid and pepsin, bile acids are other potentially harmful erosive components of gastric content. Exposure of rabbit esophageal mucosa to bile acids can cause the generation of DIS in both acidic and weakly acidic conditions [106]. This is in contrast to the earlier finding that biopsies of GERD patients with and without duodenal reflux exposure show a similar amount of DIS [97].

The concept of DIS generation in response to acid and acid-pepsin proved to hold *in vivo*, in a model where infusion of acid and acid-pepsin solutions in the distal esophagus was followed by the direct assessment of DIS in biopsy samples by TEM [107]. The concept of acid exposure generating DIS is corroborated by the fact that DIS recovered after 3 months of

acid suppressive therapy [108]. Subsequently, the effect of weakly acidic solutions and bile salts on DIS was studied and proved to be present in a similar *in vivo* model [106]. An interesting finding in this study is that although these solutions provoked DIS, the majority of subjects did not experience heartburn. This supports the hypothesis that symptom generation is multifactorial and DIS is not the only determinant of symptoms.

Next to luminal effects, there are indications that systemic factors play a role in the generation of DIS. The predominant location of DIS in the basal layer of the epithelium, and the less pronounced presence in the more directly exposed prickle cell and functional layers, suggests that circulating agents such as cytokines exert a systemic effect, possibly in response to the aggressive luminal contents. Furthermore, it has been shown that acute stress increases the perception of heartburn in GERD patients [109] and acute stress enhances the effect of acid-pepsin on DIS and the permeability to small molecules in a rat model [110].

Visceral hypersensitivity

Visceral hypersensitivity is an established concept in inflammatory and functional gastrointestinal disorders, where patients have a heightened perception of various stimuli in the gastrointestinal tract [111]. This reduced pain threshold to mechanical, chemical, thermal or electrical stimuli is considered to be caused by a combination of peripheral sensitization, central sensitization and interactions between the neural and immune systems [112]. The previously mentioned finding that stress influences patients' heartburn perception suggests a similar role for visceral hypersensitivity in the pathophysiology of GERD. Peripheral nociceptors in the esophagus express several cation channels, of which the most relevant for GERD are cation channels sensitive to a low pH, like acid-sensitive ion channels (ASICs) 1–3, ionotropic purinergic (P2X) receptors and the transient receptor potential (TRP) channels. TRPV1, a member of the TRP family, has been shown to be upregulated in the esophageal mucosa of patients with esophagitis and NERD [113,114]. Sensitization of peripheral neurons occurs once the signaling threshold of these channels reduces in response to continuous noxious stimulation. A possible mechanism of sensitization in GERD is through direct contact of these channels with H^+ by the presence of DIS and subsequent acidification of the intercellular space or via indirect signaling by cytokines released in response to the exposure of epithelium to aggressive gastric contents.

Central sensitization occurs once repetitive firing from the peripheral neurons leads to triggering of intercellular changes in the spinal dorsal horn neurons responsible for central signal transduction of nociceptors. This in turn leads to amplified responses to peripheral stimuli and also

to triggering of adjacent spinal neurons, giving rise to hypersensitivity of more remote areas such as the chest wall [115].

Sustained esophageal contractions

Another mechanism proposed as a mediator in the perception of reflux episodes is the phenomenon of sustained esophageal contractions (SEC). Using high-frequency endoscopic ultrasonography, intermittent thickening of the esophageal wall can be observed, representing a sustained contraction of the longitudinal muscle. SECs preceded 70% of heartburn symptoms during ambulatory ultrasonography combined with a pH measurement and accompanied 75% of provoked heartburn symptoms during a Bernstein test [116]. SECs were also found to correlate with symptoms in patients with unexplained chest pain [117]. The findings suggest a role of SECs in the pathophysiology of esophageal pain perception, although it should be noted that all findings were obtained in a small number of patients. Furthermore, the concept cannot explain the entire spectrum of symptom generation since the majority of SECs do not cause symptoms and 30% of heartburn symptoms are not accompanied by a SEC [116].

Genetic factors

The observation that reflux symptoms are often clustered in families prompted a search for genetic factors that might play a role in GERD. An association was found between GERD and the heterozygous genotype of the C825T allele of the G-protein B3 subunit, coding for a receptor frequently present in the neural brain-gut axis which is associated with intracellular cell transduction [118]. The polymorphism had previously been associated with visceral hypersensitivity in functional dyspepsia. The association was specifically present in patients with a "hypersensitive esophagus," suggesting a genetic predisposition to visceral hypersensitivity in GERD.

Summary

Gastroesophageal reflux disease is a multifactorial disorder and although many aspects of the pathophysiology have been described, parts remain to be elucidated. The pathophysiology comprises factors that determine the exposure of the esophageal mucosa to gastric contents, and factors that influence the esophageal sensitivity and thereby alter the perception of reflux. The esophageal exposure to gastric contents is dependent on reflux mechanisms as TLESRs, LES hypotension and the presence of an anatomical disruption of the normal anti-reflux barrier, i.e. a hiatal hernia. Additionally,

reflux is facilitated by gastric factors such as delayed emptying of the proximal stomach and an altered position of the acid pocket. Obesity leads to an increased severity of gastroesophageal reflux by influencing several of these mechanisms.

The fact that esophageal acid exposure and symptom generation are mainly independent phenomena has led to the understanding that sensitivity of the esophagus and perception of reflux are equally important in the pathophysiology of GERD. Characteristics of the reflux episode itself, such as proximal extent, duration and the composition of the refluxate, can lead to increased perception. Suggested changes at the esophageal level contributing to an increased perception of reflux are the presence of dilated intercellular spaces and visceral hypersensitivity. Lastly, genetic mutations could predispose to visceral hypersensitivity and thereby to reflux perception in GERD.

References

1 GI Motility online. Available from: www.nature.com/gimo/index.html.
2 Sloan S, Rademaker AW, Kahrilas PJ. Determinants of gastroesophageal junction incompetence: hiatal hernia, lower esophageal sphincter, or both? Ann Intern Med 1992;117(12):977–82.
3 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(6):1439–46.
4 Pouderoux P, Verdier E, Kahrilas PJ. Patterns of esophageal inhibition during swallowing, pharyngeal stimulation, and transient LES relaxation. Lower esophageal sphincter. Am J Physiol Gastrointest Liver Physiol 2003;284(2):G242–7.
5 Schoeman MN, Holloway RH. Integrity and characteristics of secondary oesophageal peristalsis in patients with gastro-oesophageal reflux disease. Gut 1995;36(4):499–504.
6 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(2):378–84.
7 Wyman JB, Dent J, Heddle R, Dodds WJ, Toouli J, Downton J. Control of belching by the lower oesophageal sphincter. Gut 1990;31(6):639–46.
8 Dent J, Dodds WJ, Friedman RH, *et al*. Mechanism of gastroesophageal reflux in recumbent asymptomatic human subjects. J Clin Invest 1980;65(2):256–67.
9 Holloway RH, Penagini R, Ireland AC. Criteria for objective definition of transient lower esophageal sphincter relaxation. Am J Physiol 1995;268(1 Pt 1):G128–33.
10 Holloway RH, Boeckxstaens GE, Penagini R, Sifrim D, Smout A, Ruth M. T1229 objective definition and detection of transient lower esophageal relaxation revisited: is there room for improvement? Gastroenterology 2009;136(5, Suppl 1):A-527.
11 Franzi SJ, Martin CJ, Cox MR, Dent J. Response of canine lower esophageal sphincter to gastric distension. Am J Physiol 1990;259(3 Pt 1):G380–5.
12 Straathof JWA, van Veen MM, Masclee AAM. Provocation of transient lower esophageal sphincter relaxations during continuous gastric distension. Scand J Gastroenterol 2002;37(10):1140–3.

13 Scheffer RCH, Akkermans LMA, Bais JE, Roelofs JMM, Smout AJPM, Gooszen HG. Elicitation of transient lower oesophageal sphincter relaxations in response to gastric distension and meal ingestion. Neurogastroenterol Motil 2002;14(6):647–55.
14 Mittal RK, Holloway RH, Penagini R, Blackshaw LA, Dent J. Transient lower esophageal sphincter relaxation. Gastroenterology 1995;109(2):601–10.
15 Mittal RK, Fisher MJ. Electrical and mechanical inhibition of the crural diaphragm during transient relaxation of the lower esophageal sphincter. Gastroenterology 1990;99(5):1265–8.
16 Shi G, Pandolfino JE, Joehl RJ, Brasseur JG, Kahrilas PJ. Distinct patterns of oesophageal shortening during primary peristalsis, secondary peristalsis and transient lower oesophageal sphincter relaxation. Neurogastroenterol Motil 2002;14(5):505–12.
17 Babaei A, Bhargava V, Korsapati H, Zheng WH, Mittal RK. A unique longitudinal muscle contraction pattern associated with transient lower esophageal sphincter relaxation. Gastroenterology 2008;134(5):1322–31.
18 Kuribayashi S, Massey BT, Hafeezullah M, *et al*. Terminating motor events for TLESR are influenced by the presence and distribution of refluxate. Am J Physiol Gastrointest Liver Physiol 2009;297(1):G71–5.
19 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(8):1034–9.
20 Van Herwaarden MA, Katzka DA, Smout AJ, Samsom M, Gideon M, Castell DO. Effect of different recumbent positions on postprandial gastroesophageal reflux in normal subjects. Am J Gastroenterol 2000;95(10):2731–6.
21 Freidin N, Fisher MJ, Taylor W, *et al*. Sleep and nocturnal acid reflux in normal subjects and patients with reflux oesophagitis. Gut 1991;32(11):1275–9.
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(4):890–6.
23 Kalia M, Mesulam MM. Brain stem projections of sensory and motor components of the vagus complex in the cat: II. Laryngeal, tracheobronchial, pulmonary, cardiac, and gastrointestinal branches. J Comp Neurol 1980;193(2):467–508.
24 Rinaman L, Card JP, Schwaber JS, Miselis RR. Ultrastructural demonstration of a gastric monosynaptic vagal circuit in the nucleus of the solitary tract in rat. J Neurosci 1989;9(6):1985–96.
25 Rossiter CD, Norman WP, Jain M, Hornby PJ, Benjamin S, Gillis RA. Control of lower esophageal sphincter pressure by two sites in dorsal motor nucleus of the vagus. Am J Physiol 1990;259(6 Pt 1):G899–906.
26 Niedringhaus M, Jackson PG, Evans SRT, Verbalis JG, Gillis RA, Sahibzada N. Dorsal motor nucleus of the vagus: a site for evoking simultaneous changes in crural diaphragm activity, lower esophageal sphincter pressure, and fundus tone. Am J Physiol Regul Integr Comp Physiol 2008;294(1):R121–31.
27 Young RL, Page AJ, Cooper NJ, Frisby CL, Blackshaw LA. Sensory and motor innervation of the crural diaphragm by the vagus nerves. Gastroenterology 2010;138(3):1091–101.
28 Yuan S, Costa M, Brookes SJ. Neuronal pathways and transmission to the lower esophageal sphincter of the guinea pig. Gastroenterology 1998;115(3):661–71.
29 Kessing BF, Conchillo JM, Bredenoord AJ, Smout AJPM, Masclee AAM. Review article: the clinical relevance of transient lower oesophageal sphincter relaxations in gastro-oesophageal reflux disease. Aliment Pharmacol Ther 2011;33(6):650–61.
30 Yuan CS, Liu D, Attele AS. GABAergic effects on nucleus tractus solitarius neurons receiving gastric vagal inputs. J Pharmacol Exp Ther 1998;286(2):736–41.

31 Blackshaw LA, Smid SD, O'Donnell TA, Dent J. GABA(B) receptor-mediated effects on vagal pathways to the lower oesophageal sphincter and heart. Br J Pharmacol 2000;130(2):279–88.
32 Cartmell J, Schoepp DD. Regulation of neurotransmitter release by metabotropic glutamate receptors. J Neurochem 2000;75(3):889–907.
33 Young RL, Page AJ, O'Donnell TA, Cooper NJ, Blackshaw LA. Peripheral versus central modulation of gastric vagal pathways by metabotropic glutamate receptor 5. Am J Physiol Gastrointest Liver Physiol 2007;292(2):G501–11.
34 Van Sickle MD, Oland LD, Ho W, *et al*. Cannabinoids inhibit emesis through CB1 receptors in the brainstem of the ferret. Gastroenterology 2001;121(4):767–74.
35 Bredenoord AJ, Weusten BLAM, Timmer R, Smout AJPM. Gastro-oesophageal reflux of liquids and gas during transient lower oesophageal sphincter relaxations. Neurogastroenterol Motil 2006;18(10):888–93.
36 Sifrim D, Holloway R. Transient lower esophageal sphincter relaxations: how many or how harmful? Am J Gastroenterol 2001;96(9):2529–32.
37 Frankhuisen R, van Herwaarden MA, Scheffer RC, Hebbard GS, Gooszen HG, Samsom M. Increased intragastric pressure gradients are involved in the occurrence of acid reflux in gastroesophageal reflux disease. Scand J Gastroenterol 2009;44(5):545–50.
38 Pandolfino JE, Shi G, Curry J, Joehl RJ, Brasseur JG, Kahrilas PJ. Esophagogastric junction distensibility: a factor contributing to sphincter incompetence. Am J Physiol Gastrointest Liver Physiol 2002;282(6):G1052–8.
39 Pandolfino JE, Shi G, Trueworthy B, Kahrilas PJ. Esophagogastric junction opening during relaxation distinguishes nonhernia reflux patients, hernia patients, and normal subjects. Gastroenterology 2003;125(4):1018–24.
40 Wu JCY, Mui LM, Cheung CMY, Chan Y, Sung JJY. Obesity is associated with increased transient lower esophageal sphincter relaxation. Gastroenterology 2007;132(3):883–9.
41 Cohen S, Harris LD. Does hiatus hernia affect competence of the gastroesophageal sphincter? N Engl J Med 1971 13;284(19):1053–6.
42 Dodds WJ, Dent J, Hogan WJ, *et al*. Mechanisms of gastroesophageal reflux in patients with reflux esophagitis. N Engl J Med 1982;307(25):1547–52.
43 Kasapidis P, Vassilakis JS, Tzovaras G, Chrysos E, Xynos E. Effect of hiatal hernia on esophageal manometry and pH-metry in gastroesophageal reflux disease. Dig Dis Sci 1995;40(12):2724–30.
44 Mattioli S, d'Ovidio F, di Simone MP, *et al*. Clinical and surgical relevance of the progressive phases of intrathoracic migration of the gastroesophageal junction in gastroesophageal reflux disease. J Thorac Cardiovasc Surg 1998;116(2):267–75.
45 Jones MP, Sloan SS, Jovanovic B, Kahrilas PJ. Impaired egress rather than increased access: an important independent predictor of erosive oesophagitis. Neurogastroenterol Motil 2002;14(6):625–31.
46 Patti MG, Goldberg HI, Arcerito M, Bortolasi L, Tong J, Way LW. Hiatal hernia size affects lower esophageal sphincter function, esophageal acid exposure, and the degree of mucosal injury. Am J Surg 1996;171(1):182–6.
47 Berstad A, Weberg R, Frøyshov Larsen I, Hoel B, Hauer-Jensen M. Relationship of hiatus hernia to reflux oesophagitis. A prospective study of coincidence, using endoscopy. Scand J Gastroenterol 1986;21(1):55–8.
48 Cameron AJ. Barrett's esophagus: prevalence and size of hiatal hernia. Am J Gastroenterol 1999;94(8):2054–9.
49 Kahrilas PJ, Lin S, Chen J, Manka M. The effect of hiatus hernia on gastro-oesophageal junction pressure. Gut 1999;44(4):476–82.

50 Bredenoord AJ, Weusten BLAM, Timmer R, Smout AJPM. Characteristics of gastro-esophageal reflux in symptomatic patients with and without excessive esophageal acid exposure. Am J Gastroenterol 2006;101(11):2470–5.
51 Mittal RK, Lange RC, McCallum RW. Identification and mechanism of delayed esophageal acid clearance in subjects with hiatus hernia. Gastroenterology 1987;92(1):130–5.
52 Sloan S, Kahrilas PJ. Impairment of esophageal emptying with hiatal hernia. Gastroenterology 1991;100(3):596–605.
53 McCallum RW, Berkowitz DM, Lerner E. Gastric emptying in patients with gastro-esophageal reflux. Gastroenterology 1981;80(2):285–91.
54 Shay SS, Eggli D, McDonald C, Johnson LF. Gastric emptying of solid food in patients with gastroesophageal reflux. Gastroenterology 1987;92(2):459–65.
55 Carmagnola S, Fraquelli M, Cantù P, Conte D, Penagini R. Relationship between acceleration of gastric emptying and oesophageal acid exposure in patients with endoscopy-negative gastro-oesophageal reflux disease. Scand J Gastroenterol 2006;41(7):767–72.
56 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(3):330–3.
57 Stacher G, Lenglinger J, Bergmann H, *et al*. Gastric emptying: a contributory factor in gastro-oesophageal reflux activity? Gut 2000;47(5):661–6.
58 Herculano JRL Jr, Troncon LE, Aprile LR, *et al*. Diminished retention of food in the proximal stomach correlates with increased acidic reflux in patients with gastro-esophageal reflux disease and dyspeptic symptoms. Dig Dis Sci 2004;49(5):750–6.
59 Fletcher J, Wirz A, Young J, Vallance R, McColl KE. Unbuffered highly acidic gastric juice exists at the gastroesophageal junction after a meal. Gastroenterology 2001;121(4):775–83.
60 Beaumont H, Bennink RJ, de Jong J, Boeckxstaens GE. The position of the acid pocket as a major risk factor for acidic reflux in healthy subjects and patients with GORD. Gut 2010;59(4):441–51.
61 Katz LC, Just R, Castell DO. Body position affects recumbent postprandial reflux. J Clin Gastroenterol 1994;18(4):280–3.
62 Wajed SA, Streets CG, Bremner CG, DeMeester TR. Elevated body mass disrupts the barrier to gastroesophageal reflux; discussion 1018-9. Arch Surg 2001;136(9):1014–18.
63 Hong D, Khajanchee YS, Pereira N, Lockhart B, Patterson EJ, Swanstrom LL. Manometric abnormalities and gastroesophageal reflux disease in the morbidly obese. Obes Surg 2004;14(6):744–9.
64 Suter M, Dorta G, Giusti V, Calmes JM. Gastro-esophageal reflux and esophageal motility disorders in morbidly obese patients. Obes Surg 2004;14(7):959–66.
65 El-Serag HB, Ergun GA, Pandolfino J, Fitzgerald S, Tran T, Kramer JR. Obesity increases oesophageal acid exposure. Gut 2007;56(6):749–55.
66 Corley DA. Obesity and the rising incidence of oesophageal and gastric adenocarcinoma: what is the link? Gut 2007;56(11):1493–4.
67 Crowell MD, Bradley A, Hansel S, *et al*. Obesity is associated with increased 48-h esophageal acid exposure in patients with symptomatic gastroesophageal reflux. Am J Gastroenterol 2009;104(3):553–9.
68 Räihä I, Impivaara O, Seppälä M, Knuts LR, Sourander L. Determinants of symptoms suggestive of gastroesophageal reflux disease in the elderly. Scand J Gastroenterol 1993;28(11):1011–14.
69 Chang CS, Poon SK, Lien HC, Chen GH. The incidence of reflux esophagitis among the Chinese. Am J Gastroenterol 1997;92(4):668–71.

70 Jaffin BW, Knoepflmacher P, Greenstein R. High prevalence of asymptomatic esophageal motility disorders among morbidly obese patients. Obes Surg 1999;9(4):390–5.
71 Lagergren J, Bergström R, Nyrén O. No relation between body mass and gastro-oesophageal reflux symptoms in a Swedish population based study. Gut 2000;47(1):26–9.
72 Talley NJ, Howell S, Poulton R. Obesity and chronic gastrointestinal tract symptoms in young adults: a birth cohort study. Am J Gastroenterol 2004;99(9):1807–14.
73 Blondeau K, van Oudenhove L, Farré R, *et al*. T1682 increasing body weight is associated with a higher incidence and proximal extent of reflux in patients with GERD Both 'on' and 'off' PPI therapy. Gastroenterology 2010;138(5, Suppl 1):S-556.
74 O'Brien TF Jr. Lower esophageal sphincter pressure (LESP) and esophageal function in obese humans. J Clin Gastroenterol 1980;2(2):145–8.
75 Stene-Larsen G, Weberg R, Frøyshov Larsen I, Bjørtuft O, Hoel B, Berstad A. Relationship of overweight to hiatus hernia and reflux oesophagitis. Scand J Gastroenterol 1988;23(4):427–32.
76 Wilson LJ, Ma W, Hirschowitz BI. Association of obesity with hiatal hernia and esophagitis. Am J Gastroenterol 1999;94(10):2840–4.
77 Pandolfino JE, El-Serag HB, Zhang Q, Shah N, Ghosh SK, Kahrilas PJ. Obesity: a challenge to esophagogastric junction integrity. Gastroenterology 2006;130(3):639–49.
78 Hampel H, Abraham NS, El-Serag HB. Meta-analysis: obesity and the risk for gastroesophageal reflux disease and its complications. Ann Intern Med 2005 2; 143(3):199–211.
79 Jacobson BC, Somers SC, Fuchs CS, Kelly CP, Camargo CA Jr. Body-mass index and symptoms of gastroesophageal reflux in women. N Engl J Med 2006;354(22):2340–8.
80 El-Serag HB, Johanson JF. Risk factors for the severity of erosive esophagitis in Helicobacter pylori-negative patients with gastroesophageal reflux disease. Scand J Gastroenterol 2002;37(8):899–904.
81 Kjellin A, Ramel S, Rössner S, Thor K. Gastroesophageal reflux in obese patients is not reduced by weight reduction. Scand J Gastroenterol 1996;31(11):1047–51.
82 Mathus-Vliegen EMH, van Weeren M, van Eerten PV. Los function and obesity: the impact of untreated obesity, weight loss, and chronic gastric balloon distension. Digestion 2003;68(2–3):161–8.
83 De Groot NL, Burgerhart JS, van de Meeberg PC, de Vries DR, Smout AJPM, Siersema PD. Systematic review: the effects of conservative and surgical treatment for obesity on gastro-oesophageal reflux disease. Aliment Pharmacol Ther 2009;30(11–12):1091–102.
84 Weusten BL, Roelofs JM, Akkermans LM, van Berge-Henegouwen GP, Smout AJ. The symptom-association probability: an improved method for symptom analysis of 24-hour esophageal pH data. Gastroenterology 1994;107(6):1741–5.
85 Colas-Atger E, Bonaz B, Papillon E, *et al*. Relationship between acid reflux episodes and gastroesophageal reflux symptoms is very inconstant. Dig Dis Sci 2002;47(3):645–51.
86 Bredenoord AJ, Weusten BLAM, Curvers WL, Timmer R, Smout AJPM. Determinants of perception of heartburn and regurgitation. Gut 2006;55(3):313–18.
87 Weusten BL, Akkermans LM, van Berge-Henegouwen GP, Smout AJ. Symptom perception in gastroesophageal reflux disease is dependent on spatiotemporal reflux characteristics. Gastroenterology 1995;108(6):1739–44.
88 Smith JL, Opekun AR, Larkai E, Graham DY. Sensitivity of the esophageal mucosa to pH in gastroesophageal reflux disease. Gastroenterology 1989;96(3):683–9.
89 Hemmink GJM, Bredenoord AJ, Weusten BLAM, Monkelbaan JF, Timmer R, Smout AJPM. Esophageal pH-impedance monitoring in patients with therapy-resistant reflux symptoms: "on" or "off" proton pump inhibitor? Am J Gastroenterol 2008;103(10):2446–53.

90 Mainie I, Tutuian R, Shay S, *et al*. Acid and non-acid reflux in patients with persistent symptoms despite acid suppressive therapy: a multicentre study using combined ambulatory impedance-pH monitoring. Gut 2006;55(10):1398–402.
91 Emerenziani S, Sifrim D, Habib FI, *et al*. Presence of gas in the refluxate enhances reflux perception in non-erosive patients with physiological acid exposure of the oesophagus. Gut 2008;57(4):443–7.
92 Logan KR, Hopwood D, Milne G. Cellular junctions in human oesophageal epithelium. J Pathol 1978;126(3):157–63.
93 Ismail-Beigi F, Horton PF, Pope CE. Histological consequences of gastroesophageal reflux in man. Gastroenterology 1970;58(2):163–74.
94 Hopwood D, Milne G, Logan KR. Electron microscopic changes in human oesophageal epithelium in oesophagitis. J Pathol 1979;129(4):161–7.
95 Tobey NA, Carson JL, Alkiek RA, Orlando RC. Dilated intercellular spaces: a morphological feature of acid reflux-damaged human esophageal epithelium. Gastroenterology 1996;111(5):1200–5.
96 Barlow WJ, Orlando RC. The pathogenesis of heartburn in nonerosive reflux disease: a unifying hypothesis. Gastroenterology 2005;128(3):771–8.
97 Calabrese C, Fabbri A, Bortolotti M, *et al*. Dilated intercellular spaces as a marker of oesophageal damage: comparative results in gastro-oesophageal reflux disease with or without bile reflux. Aliment Pharmacol Ther 2003;18(5):525–32.
98 Caviglia R, Ribolsi M, Gentile M, *et al*. Dilated intercellular spaces and acid reflux at the distal and proximal oesophagus in patients with non-erosive gastro-oesophageal reflux disease. Aliment Pharmacol Ther 2007;25(5):629–36.
99 Van Malenstein H, Farré R, Sifrim D. Esophageal dilated intercellular spaces (DIS) and nonerosive reflux disease. Am J Gastroenterol 2008;103(4):1021–8.
100 Villanacci V, Grigolato PG, Cestari R, *et al*. Dilated intercellular spaces as markers of reflux disease: histology, semiquantitative score and morphometry upon light microscopy. Digestion 2001;64(1):1–8.
101 Zentilin P, Savarino V, Mastracci L, *et al*. Reassessment of the diagnostic value of histology in patients with GERD, using multiple biopsy sites and an appropriate control group. Am J Gastroenterol 2005;100(10):2299–306.
102 Solcia E, Villani L, Luinetti O, *et al*. Altered intercellular glycoconjugates and dilated intercellular spaces of esophageal epithelium in reflux disease. Virchows Arch 2000;436(3):207–16.
103 Cui R, Zhou L, Lin S, *et al*. The feasibility of light microscopic measurements of intercellular spaces in squamous epithelium in the lower-esophagus of GERD patients. Dis Esophagus 2011;24(1):1–5.
104 Tobey NA, Hosseini SS, Argote CM, Dobrucali AM, Awayda MS, Orlando RC. Dilated intercellular spaces and shunt permeability in nonerosive acid-damaged esophageal epithelium. Am J Gastroenterol 2004;99(1):13–22.
105 Tobey NA, Hosseini SS, Caymaz-Bor C, Wyatt HR, Orlando GS, Orlando RC. The role of pepsin in acid injury to esophageal epithelium. Am J Gastroenterol 2001;96(11):3062–70.
106 Farré R, Fornari F, Blondeau K, *et al*. Acid and weakly acidic solutions impair mucosal integrity of distal exposed and proximal non-exposed human oesophagus. Gut 2010;59(2):164–9.
107 Bove M, Vieth M, Dombrowski F, Ny L, Ruth M, Lundell L. Acid challenge to the human esophageal mucosa: effects on epithelial architecture in health and disease. Dig Dis Sci 2005;50(8):1488–96.

108 Calabrese C, Bortolotti M, Fabbri A, *et al*. Reversibility of GERD ultrastructural alterations and relief of symptoms after omeprazole treatment. Am J Gastroenterol 2005;100(3):537–42.
109 Fass R, Naliboff BD, Fass SS, *et al*. The effect of auditory stress on perception of intraesophageal acid in patients with gastroesophageal reflux disease. Gastroenterology 2008;134(3):696–705.
110 Farré R, de Vos R, Geboes K, *et al*. Critical role of stress in increased oesophageal mucosa permeability and dilated intercellular spaces. Gut 2007;56(9):1191–7.
111 Anand P, Aziz Q, Willert R, van Oudenhove L. Peripheral and central mechanisms of visceral sensitization in man. Neurogastroenterol Motil 2007;19(1 Suppl):29–46.
112 Knowles CH, Aziz Q. Visceral hypersensitivity in non-erosive reflux disease. Gut 2008;57(5):674–83.
113 Bhat YM, Bielefeldt K. Capsaicin receptor (TRPV1) and non-erosive reflux disease. Eur J Gastroenterol Hepatol 2006;18(3):263–70.
114 Guarino MPL, Cheng L, Ma J, *et al*. Increased TRPV1 gene expression in esophageal mucosa of patients with non-erosive and erosive reflux disease. Neurogastroenterol Motil 2010;22(7):746–51.
115 Sarkar S, Aziz Q, Woolf CJ, Hobson AR, Thompson DG. Contribution of central sensitisation to the development of non-cardiac chest pain. Lancet 2000;356(9236):1154–9.
116 Pehlivanov N, Liu J, Mittal RK. Sustained esophageal contraction: a motor correlate of heartburn symptom. Am J Physiol Gastrointest Liver Physiol 2001;281(3):G743–51.
117 Balaban DH, Yamamoto Y, Liu J, *et al*. Sustained esophageal contraction: a marker of esophageal chest pain identified by intraluminal ultrasonography. Gastroenterology 1999;116(1):29–37.
118 De Vries DR, ter Linde JJM, van Herwaarden MA, Smout AJPM, Samsom M. Gastroesophageal reflux disease is associated with the C825T polymorphism in the G-protein beta3 subunit gene (GNB3). Am J Gastroenterol 2009;104(2):281–5.