

# Chapter 2

## The Pathophysiology of Gastroesophageal Reflux

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### Gastroesophageal Reflux

The backflow of gastric contents into the esophagus, gastroesophageal reflux (GER), is a normal physiological phenomenon that occurs in most people, particularly after meals. Brief and infrequent exposure of the esophagus to gastric contents does not result in injury and disease, implying that there are intrinsic defense mechanisms that act to maintain mucosal integrity. In fact, based upon pH monitoring studies, up to 50 reflux episodes a day (below pH 4) are considered normal. However, esophageal symptoms and complications arise when reflux is prolonged and/or there is a breakdown in the defense mechanisms. When reflux is in excess, heartburn is experienced, described as a burning sensation behind the sternum. Most people experience heartburn from time to time. Patients with long-term GER may develop esophagitis (inflammation of the esophagus) thought to occur when the normal defense mechanisms break down. This is termed gastroesophageal reflux disease (GERD). GERD is accepted as possibly the most common chronic disease of adults in the USA, affecting more than 30% of Western society [1, 2].

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GER is a major factor in the development of Barrett's esophagus in which the esophageal epithelia are changed from stratified squamous to pseudostratified columnar intestinal-type epithelium. Approximately 10% of patients with esophagitis develop Barrett's esophagus. Barrett's esophagus is a premalignant condition with an associated risk of developing esophageal adenocarcinoma, with reflux playing a major role [3].

## **Laryngopharyngeal Reflux**

When gastric reflux travels more proximally into the laryngopharynx, it is termed laryngopharyngeal reflux (LPR) [4]. Other terms, such as gastropharyngeal reflux (GPR) and esophagopharyngeal reflux (EPR), have been used synonymously. These are all considered as part of extra-esophageal reflux (EER), reflux involving structures other than, or in addition to, the esophagus. LPR contributes to several otolaryngologic symptoms, inflammatory disorders, and perhaps also neoplastic diseases of the laryngopharynx [4–9] and appears to be as common in children and infants as in adults [10]. It is estimated that 10% of patients visiting otolaryngology clinics have reflux-attributed disease, and up to 55% of patients with hoarseness have reflux into their laryngopharynx [4, 8]. LPR is actually one of the most common factors associated with inflammation in the upper airways. Compared to the esophagus, where up to 50 reflux episodes (below pH 4) a day are considered normal or physiologic, the laryngopharynx and other extra-esophageal structures appear to be more sensitive to injury by gastric refluxate. It has been shown experimentally that three or less reflux episodes per week into the laryngopharynx result in severe laryngeal damage [4, 11].

Symptoms of LPR include dysphonia, throat clearing, postnasal drip, chronic cough, dysphagia, globus pharyngeus, excessive phlegm, heartburn, dyspnea, laryngospasm, and wheezing [12]. Signs of LPR are commonly associated with posterior laryngitis and include erythema and edema of the larynx, especially in the post-cricoid and hypopharyngeal areas. Additionally, signs of chronic laryngitis including vocal fold edema, diffuse laryngeal erythema and edema, pseudosulcus vocalis, obliteration of the laryngeal ventricles, thickened laryngopharyngeal mucus, and mucosal granulomata are commonly associated with LPR [12].

## **Review of Gastric Reflux Contents and Their Role in Reflux Disease**

### ***Gastric Acid***

Gastric acid is produced by parietal cells in fundic glands of the gastric mucosa. Parietal cells contain an extensive secretory network, called canaliculi, from which

gastric acid is secreted into the lumen of the stomach. Chloride and hydrogen ions are secreted separately from the cytoplasm of parietal cells and mixed in the canaliculi. Gastric acid is then secreted into the lumen of the oxyntic gland and gradually reaches the stomach lumen. Gastric acid is approximately pH 2 in the human stomach lumen, the acidity being maintained by the proton pump  $H^+/K^+$  ATPase. The resulting highly acidic environment in the stomach lumen causes proteins from food to denature, exposing the protein's peptide bonds. The chief cells of the stomach secrete inactive enzymes, pepsinogen and renin, for protein breakdown. Gastric acid then activates pepsinogen into pepsin, which enzymatically aids digestion by breaking the bonds linking amino acids, a process known as proteolysis. In addition, the acidic environment of the stomach inhibits the growth of many microorganisms, thereby controlling the gut's bacterial load to help prevent infection.

In hypochlorhydria and achlorhydria, there is low or no gastric acid in the stomach, potentially leading to problems as the disinfectant properties of the gastric lumen are decreased. In such conditions, there is greater risk of infections of the digestive tract (such as infection with *Helicobacter* bacteria). In Zollinger–Ellison syndrome and hypercalcemia, there are increased gastrin levels, leading to excess gastric acid production, which can cause gastric ulcers. Reflux of gastric acid into the esophagus and airway also causes injury and disease. Erosive esophagitis and heartburn are consequences of abnormal gastric acid exposure [13]. Intraesophageal perfusion experiments with acidic solutions demonstrated symptoms of heartburn at pH 6 and below [14]. Combined multichannel intraluminal impedance—pH monitoring studies have shown a significant correlation between both acid and nonacid reflux episodes and heartburn [15–17].

Dilated intracellular spaces are associated with impaired mucosal integrity and are detected in patients with GER as well as in animal models and healthy volunteers exposed to acidic contents [18–20]. The mucosal immune response and presence of specific inflammatory mediators, such as interleukins IL-8 and IL-6, are well characterized in GERD [1].

The  $H^+/K^+$  pump in the stomach is the target of proton pump inhibitors, used to increase gastric pH to treat reflux disease. Histamine  $H_2$  antagonists indirectly decrease gastric acid production, and antacids neutralize existing acid.

## *Pepsin*

Pepsin is expressed as a pro-form zymogen, pepsinogen, whose primary structure has an additional 44 amino acids. Chief cells in the stomach release the zymogen, pepsinogen. This zymogen is activated by hydrochloric acid (HCl), which is released from parietal cells in the stomach lining. The hormone, gastrin, and the vagus nerve trigger the release of both pepsinogen and HCl from the stomach lining when food is ingested. Hydrochloric acid creates an acidic environment, which allows pepsinogen to unfold and cleave itself in an autocatalytic fashion, thereby generating pepsin, the active form of the enzyme. Pepsin then cleaves the 44 amino acids from pepsinogen

creating more pepsin. Pepsin will digest up to 20% of ingested amide bonds by cleaving preferentially after the N-terminus of aromatic amino acids such as phenylalanine, tryptophan, and tyrosine. Pepsin exhibits preferential cleavage for hydrophobic, preferably aromatic, residues in P1 and P1' positions. Increased susceptibility to hydrolysis occurs if there is a sulfur-containing amino acid close to the peptide bond, which has an aromatic amino acid. Pepsin cleaves Phe-Val, Gln-His, Glu-Ala, Ala-Leu, Leu-Tyr, Tyr-Leu, Gly-Phe, Phe-Phe, and Phe-Tyr bonds in the B chain of insulin. Peptides may be further digested by other proteases in the duodenum and eventually absorbed by the body. Pepsin is stored as pepsinogen so it will only be released when needed and not digest the body's own proteins in the stomach's lining.

Pepsin is maximally active at pH 2 but has activity up to pH 6.5. While inactive at pH 6.5 and above, it remains stable to pH 8. The enzyme is not irreversibly inactivated (denatured) until the ambient pH reaches 8 [21, 22]. While the stomach is designed to resist damage by pepsin, reflux of pepsin into the esophagus and laryngopharynx causes damage even above pH 4. Pepsin is considered an important etiological factor in reflux disease of the aerodigestive tract and a biomarker for reflux, whose levels and acidity can be related to the severity of damage. Pepsin compromises cell membrane integrity by disrupting the intracellular junction complexes and increasing intracellular space [23]. Pepsin also increases esophageal tissue permeability by increasing cell influx of H<sup>+</sup> ions, K<sup>+</sup> ions, and glucose and by decreasing the recovery of titrated water, causing cellular disruption [24].

## ***Bile***

Bile is produced by hepatocytes in the liver, draining through the many bile ducts that penetrate the liver. During this process, the epithelial cells add a watery solution that is rich in bicarbonates that dilutes and increases alkalinity of the solution. Bile then flows into the common hepatic duct, which joins with the cystic duct from the gallbladder to form the common bile duct. The common bile duct in turn joins with the pancreatic duct to empty into the duodenum. If the sphincter of Oddi is closed, bile is prevented from draining into the intestine and instead flows into the gallbladder, where it is stored and concentrated to up to five times its original potency between meals. This concentration occurs through the absorption of water and small electrolytes, while retaining all the original organic molecules. Cholesterol is also released with the bile, dissolved in the acids and fats found in the concentrated solution. When food is released by the stomach into the duodenum in the form of chyme, the duodenum releases cholecystokinin, which causes the gallbladder to release the concentrated bile into the duodenum to complete digestion.

Bile acts to some extent as a surfactant, helping to emulsify the fats in food. Bile salt anions have both a hydrophilic and hydrophobic site and therefore tend to aggregate around droplets of fat (triglycerides and phospholipids) to form micelles, with the hydrophobic sides toward the fat and hydrophilic to the outside. The hydrophilic

sides are positively charged due to the lecithin and other phospholipids that comprise bile, and this charge prevents fat droplets coated with bile from reaggregating into larger fat particles.

The dispersion of food fat into micelles thus provides an increased surface area for the action of the enzyme, pancreatic lipase, which digests the triglycerides and is able to reach the fatty core through gaps between the bile salts. A triglyceride is broken down into two fatty acids and a monoglyceride, which are absorbed by the villi on the intestine walls. After being transferred across the intestinal membrane, fatty acids are reformed into triglycerides, then absorbed into the lymphatic system through lacteals. Without bile salts, most of the lipids in food would be passed out in feces, undigested.

The alkaline bile also has the function of neutralizing any excess stomach acid before it enters the ileum, the final section of the small intestine. Bile salts also act as bactericides, destroying many of the microbes that may be present in ingested food.

Reflux of bile is known to cause esophagitis and is likely to play a role in extra-esophageal reflux disease [25]. The mechanism of bile-induced mucosal injury is thought to be related to “intramucosal trapping” of bile acids that results in mucosal damage primarily by disorganizing membrane structure or interfering with cellular metabolism. Taurocholic acid does this at pH 2, and chenodeoxycholic acid at pH 7. Some bile acids cause damage at high pH due to their lipophilic properties at the pH at or near the  $pK_a$  value, for example, taurocholic acid (which is a conjugated bile acid with a  $pK_a \sim 2$ ) and chenodeoxycholic acid (which is an unconjugated acid with  $pK_a \sim 7$ ) are unionized and therefore can enter the cell at an acidic and neutral pH, respectively. Once inside the cell, bile acids are trapped by ionization and subsequently cause mucosal damage.

## *Trypsin*

Trypsin is secreted into the duodenum, where it acts to hydrolyze peptides into their smaller building blocks—amino acids (these peptides are the result of the enzyme pepsin breaking down the proteins in the stomach). This enables the uptake of protein in the food because peptides (though smaller than proteins) are too big to be absorbed through the lining of the ileum. Trypsin catalyzes the hydrolysis of peptide bonds. They have an optimal operating pH of about 8 and optimal operating temperature of about 37°C.

Trypsin is produced in the pancreas in the form of an inactive zymogen, trypsinogen. When the pancreas is stimulated by cholecystokinin, trypsinogen is then secreted into the small intestine. Once in the small intestine, the enzyme, enteropeptidase, activates it into trypsin by proteolytic cleavage. The resulting trypsins themselves activate more trypsinogens (autocatalysis), so only a small amount of enteropeptidase is necessary to start the reaction. This activation mechanism is common for most serine proteases and serves to prevent autodigestion of the pancreas.

Studies have shown that trypsin can stimulate the production of inflammatory mediators. Chemokines and prostaglandins are increased in human esophageal epithelial cells when exposed to trypsin in vitro [26]. Trypsin has also been shown to induce chronic esophageal inflammation in rats [27].

## **Esophageal Defense Mechanisms to Protect Against Reflux**

The integrity of the human esophageal mucosa depends on several defense mechanisms that protect it against luminal aggressive factors such as gastric reflux. These protective mechanisms can be classified as pre-epithelial, epithelial, and post-epithelial defenses [28].

### ***Pre-epithelial Defense Mechanisms***

#### **Lower Esophageal Sphincter**

The lower esophageal sphincter (LES), detected by the presence of elevated pressure in the most distal esophageal segment relative to intragastric pressure, is an important factor preventing reflux of gastric contents into the esophageal lumen. It has been shown that physiological acid reflux episodes occur during periods of transient LES relaxations in healthy individuals [29]. Patients with reflux disease often have an increased frequency of transient LES relaxations [30, 31]. Hypotension of the LES has also been demonstrated to play a fundamental role in LPR [32].

#### **Peristalsis**

Peristalsis is the mechanism by which food boli are transported through the esophagus to the stomach. This process also plays an important role in clearing acid from the esophageal lumen. It has been shown that decreased peristalsis is responsible for impaired acid clearance in one third of patients with GERD [33].

#### **Saliva**

Saliva is secreted by the major (parotid, submandibular sublingual) and minor salivary glands. Saliva contains bicarbonate ions, which are thought to be produced by carbonic anhydrase (CA) (isoform II) present in these glands [34]. Although both CAII and CAVI isoenzymes are localized in the secretory granules of the serous acinar cells in the parotid and submandibular glands, only CAVI is secreted into the saliva. It is thought that CAII supplies the saliva with bicarbonate, while CAVI

present in the saliva is responsible for removing acid via the hydration of carbon dioxide [34], thus forming a mutually complementary system regulating salivary pH. Studies have shown that the secretory glycoprotein CAVI is significantly reduced in patients with esophagitis and esophageal ulcers [35].

Saliva is normally secreted at 0.5 ml/min, but this rate doubles during mechanical or chemical stimulation of the esophagus [36]. Aspiration of saliva has been shown to significantly increase esophageal acid clearance time and to lower luminal pH below 4 [37]. Besides bicarbonate, saliva contains epidermal growth factor (EGF) produced in the submaxillary ductal cells. EGF has been shown to enhance wound healing and decrease the permeability of the esophageal mucosa to hydrogen ions [38, 39].

### *Epithelial Defense Mechanisms*

Unlike the stomach and duodenum, the esophagus does not appear to have a well-defined surface mucous layer. Thus, if pre-epithelial defenses fail to inactivate the injurious components of the gastric refluxate, the surface epithelium will be exposed to these components. The esophageal epithelium maintains its integrity by both structural and functional defenses. Structural defenses include the physical barrier of cell membranes and intercellular junctions. Functional defenses include intracellular buffers,  $\text{Na}^+/\text{H}^+$  and  $\text{Na}^+$ -dependent  $\text{Cl}^-/\text{HCO}_3^-$  exchange systems.

#### **Structural Defenses**

Surface epithelial cells are surrounded by a glycocalyx that contains both neutral and acidic mucosubstances derived from membrane-coating granules. Acidic mucosubstances are more abundant in the superficial cell layers, where they are thought to provide a barrier against the gastric refluxate.

Cell adhesion molecules and intermediate filaments play an important role in maintaining epithelial integrity. Both desmosomes and hemidesmosomes have been identified in the human esophageal epithelium. These cell-to-cell and cell-to-matrix adhesion complexes have been shown to play an important role in epithelial defense. Hemidesmosomes mediate adhesion of epithelial cells to the basement membrane and connect the cytoskeleton to the extracellular matrix (ECM). These structures are present in the basal cell layer of the esophageal stratified squamous epithelium. A family of transmembrane receptors called integrins mediates the interaction between cells and the ECM. The  $\alpha 6\beta 4$  integrin, a major component on hemidesmosomes, can transduce signals from the ECM to the interior of the cell to organize the cytoskeleton and induce proliferation, apoptosis, or differentiation [40]. Desmosomes can also be found in the esophageal epithelium, particularly in the prickle cell layer where they often form “desmosome fields” [41]. Desmosomes bind cells together and link intermediate filament networks thus conferring structural continuity and tensile strength. The desmosomal glycoproteins are members of the cadherin family.

Cell-to-cell interactions in the esophageal epithelium are mediated by E-cadherin in a  $\text{Ca}^{2+}$ -dependent manner [42].

As basal epithelial cells mature they migrate to form the prickle and functional cell layers, before being sloughed off into the lumen. This process results in the constant renewal of esophageal epithelial cells maintaining tissue integrity, which would not be possible without modulating cell-to-basement membrane and cell-to-cell adhesion. This is supported by the finding that integrins are present in a gradient of decreasing intensity as cells move toward the lumen, perhaps allowing shedding to occur [43].

### **Epidermal Growth Factor**

EGF is a 6 kDa, 53 amino acid polypeptide that interacts with target cells by binding to a 170-kDa transmembrane receptor. The EGF receptor (EGF-R) has three distinct regions: an extracellular ligand-binding domain, a single hydrophobic transmembrane domain, and a cytoplasmic domain that possesses tyrosine kinase activity (reviewed by Jankowski et al.) [44]. Binding of EGF to its receptor results in clustering of the ligand–receptor complexes in clathrin-coated pits, receptor dimerization, auto-phosphorylation, and subsequent activation of signal transduction pathways [45]. These downstream signaling pathways include activation of tyrosine kinase activity with subsequent activation of phospholipase-C- $\gamma$ 1, which induces cell motility following phosphorylation of the E-cadherin–catenin complex [46–48]. Activation of tyrosine kinase can also result in the activation of protein kinase C, which induces mitogenesis [46].

EGF is thought to play an important role in maintaining esophageal mucosal integrity and rapid epithelial regeneration. Studies have shown that patients with low salivary EGF levels are predisposed to severe esophageal damage if they develop GERD [49, 50]. Salivary EGF has also been found to be significantly decreased in patients with LPR compared to a control group [51].

The esophagus secretes salivary EGF from submucosal glands present in the lamina propria. It has been demonstrated that exposure of the esophageal mucosa to acid and pepsin increases salivary EGF and bicarbonate output from the salivary glands [52–55], which are involved in restoration of mucosal integrity and wound healing [56].

### **Transforming Growth Factor**

The human esophagus also synthesizes transforming growth factor alpha (TGF- $\alpha$ ) [57], which shares sequence homology with EGF [58]. TGF- $\alpha$  can interact with target cells by binding to the EGF-R. In vitro and in vivo studies have shown that both EGF and TGF- $\alpha$  can promote proliferation and differentiation of epithelial cells [59]. TGF- $\alpha$  mRNA is differentially expressed compared to EGF. TGF- $\alpha$  is predominantly expressed in the superficial cell layers [57]. The expression of both

EGF and TGF- $\alpha$  in the esophageal epithelium is intriguing, as both exert similar biological effects. The physiological significance of TGF- $\alpha$  is unknown; however, it is hypothesized that both of these growth factors are involved in defense and restitution following mucosal injury.

### Carbonic Anhydrase

Carbonic anhydrase (CA) catalyzes the reversible hydration of carbon dioxide [60–62] in the reaction:



The bicarbonate ions produced are actively pumped out of the cell, via anion exchange, into the extracellular space where they can neutralize refluxed gastric acid and therefore indirectly reduce peptic activity. Eleven catalytically active isoenzymes have been isolated to date, each showing differences in activity, susceptibility to inhibitors, and tissue-specific distribution. The esophagus has been shown to express CAI–IV in the epithelium [63]. The high-activity isoenzymes CAII and IV are localized in the suprabasal cell layers, while low-activity CAI and III are in the basal cell layers. The presence of CA in the esophagus is important because endogenous bicarbonate production is capable of increasing the low pH environment, as a result of a reflux episode, from 2.5 to almost neutrality [63–65]. Patients with GERD have increased expression of CAIII in inflamed esophageal mucosa with a relocalization of expression in the basal and lower prickle cell layers in normal esophageal tissue to the suprabasal layer (which would be in contact with gastric refluxate) in inflamed tissue [28]. These findings suggest that the expression of CAIII is modified in patients with GERD as a potential protective mechanism to increase the buffering capacity of the epithelium. In contrast, patients with LPR neither show an upregulation or a redistribution [66], implying that low levels of CA isoenzymes could contribute to the pathogenesis of LPR. As already mentioned, studies have shown that the amount of CAVI, a secretory glycoprotein found in saliva, is significantly reduced in patients with esophageal and other gastric disorders [35].

### Na<sup>+</sup>/H<sup>+</sup> Exchanger

In addition to the buffering capacity from the production of HCO<sub>3</sub><sup>-</sup> by carbonic anhydrase, a Na<sup>+</sup>/H<sup>+</sup> exchanger has been demonstrated that maintains intracellular pH by removing H<sup>+</sup> ions from the cells [67–69]. Intracellular H<sup>+</sup> ions are exchanged for extracellular Na<sup>+</sup> via an ATPase [70–72]. Osmotic balance is maintained by active extrusion of Na<sup>+</sup> ions via a Na<sup>+</sup>/K<sup>+</sup> ATPase [73, 74].

### **Na<sup>+</sup>-dependent Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> Exchanger**

A Na<sup>+</sup>-dependent Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchanger has been demonstrated in rabbit esophageal cells [20, 69]. Intracellular Cl<sup>-</sup> is exchanged for extracellular HCO<sub>3</sub><sup>-</sup> to neutralize H<sup>+</sup> ions. In return, a Na<sup>+</sup>-independent Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchanger, also identified by Tobey et al. [69], prevents the pH rising to alkaline levels. Future work identifying the presence of such exchangers in human esophageal and laryngeal epithelial cells is required.

### **Heat Shock Proteins**

The esophageal epithelium regularly experiences thermal stress, and as a result heat shock proteins are produced [28]. This stress response pathway provides increased cellular protection by inducing thermotolerance to subsequent heat shock. If cells are subjected to a sublethal heat shock and allowed to recover, they can survive a second heat shock that would otherwise be lethal [75]. The majority of these stress-induced proteins are constitutively expressed and are thought to function as molecular chaperones, enabling cellular proteins to remain correctly folded and be transported to the correct destination [76]. Heat shock proteins are also thought to protect cytoskeletal structures [77, 78]. Furthermore, they have been shown to act as proteases to destroy damaged proteins that would otherwise cause the cell to undergo apoptosis [77]. Interestingly, induction of heat shock proteins has also been shown by a variety of agents other than heat, such as ethanol and acid [75, 79]. Thus many investigators now refer to these proteins more generally as the stress proteins. Yagui-Beltran et al. [79] revealed an induction of the squamous epithelium stress protein (SEP70) in cultured cells exposed to low pH, suggesting a role for this protein in acid-mediated stress response. Furthermore, the authors noted a reduction in SEP70 in samples of Barrett's esophagus. Absence of this stress-induced protein may contribute to the increased damage caused by acid reflux in patients with Barrett's esophagus. It has been shown that patients with LPR have depleted stress protein levels [80]. Furthermore, the authors demonstrated depletion of these stress proteins by pepsin in vitro. An altered stress response in LPR patients may lead to cellular injury and thus play a role in the development of disease.

### ***Post-epithelial Defense Mechanisms***

This line of defense is largely dependent on the blood supply, which delivers bicarbonate produced by erythrocyte carbonic anhydrase [81] that may neutralize refluxed acid. An increase in blood flow has been reported following exposure to low pH and bile acids [82–84].

## Pathophysiology of GERD

Intermittent exposure of the esophagus to gastric refluxate does not result in the development of disease in the majority of the population because the defense mechanisms described above act to maintain mucosal integrity. In fact, based upon pH monitoring studies, up to 50 reflux episodes per day (below pH 4) are considered normal. It is proposed that inflammation and Barrett's esophagus arise when there is a breakdown in these defense mechanisms.

## Pathophysiology of LPR

Gastric refluxate, containing acid, pepsin, bile, and trypsin, obviously passes through the esophagus (GER) to enter the laryngopharynx (LPR), yet patients with laryngeal symptoms and injury do not often have esophageal symptoms or injury. It is thought that LPR patients have intact esophageal defense mechanisms that prevent esophageal injury by the refluxate. For example, in the esophagus, peristaltic motility helps clear the refluxate, salivary bicarbonate neutralizes the refluxate, and mucous secretions prevent the refluxate from penetrating the epithelium. In addition, the upper esophageal sphincter (UES) closes to prevent refluxate from entering the laryngopharynx. It is thought that UES function is defective in many patients with reflux-attributed laryngeal symptoms and endoscopic findings. The larynx lacks many of the intrinsic defense mechanisms present in their esophageal counterparts such as peristalsis and salivary bicarbonate, perhaps explaining its increased sensitivity to gastric refluxate compared to the esophagus.

Reflux is thought to cause laryngeal symptoms and pathology by several different mechanisms. First, by direct contact of acid and pepsin with the epithelium—the microaspiration theory [5]. Second, the trauma theory suggests that exposure of the laryngeal epithelium to gastric refluxate alone is not sufficient to cause injury and that an additional factor, such as vocal abuse or concomitant viral infection, is necessary to cause mucosal lesions [4, 85]. Finally, the esophageal–bronchial reflex theory suggests that acid in the distal esophagus stimulates vagally mediated reflexes that cause chronic cough, which in turn causes laryngeal symptoms and lesions [86].

## Differences Between LPR and GERD

Ossakow et al. compared symptoms of patients with reflux esophagitis with those of patients with laryngitis [87]. They found that hoarseness was the most prevalent symptom of patients with LPR (100%), although none of the patients with GERD reported experiencing hoarseness. Heartburn was present in the majority of patients

with GERD (89%), but only a small percentage of LPR patients (6%). Wiener et al. demonstrated abnormal pH studies in 78% of patients with hoarseness. Despite abnormal pH studies, all had normal esophageal manometry and 72% had normal endoscopy with biopsy [88]. This supports the theory that the majority of LPR patients have normal acid clearance mechanisms [32, 89]. Although both esophagitis and laryngitis are likely to be caused by the damaging effects of the corrosive refluxate, significantly more damage occurs in the larynx compared to the esophagus following exposure to acid and pepsin. This may be because the esophagus has better defense mechanisms to counteract such damage, for example, peristalsis, saliva, and bicarbonate production [4, 23]. Therefore, although there may not be sufficient reflux to cause esophagitis, it may be enough to develop symptomatic LPR.

The pattern and mechanism of LPR and GERD are different. LPR patients typically have upright (daytime) reflux with good esophageal motor function and no esophagitis, whereas GERD patients have supine (nocturnal) reflux and esophageal dysmotility [4, 90, 91]. GERD patients often have a dysfunction of the lower esophageal sphincter, while the upper esophageal sphincter has been reported to be a primary defect in LPR patients [92]. The different pattern and mechanism of reflux may explain the different manifestations observed in LPR and GERD.

## **Role of Nonacid Reflux in Laryngeal Inflammation Disease**

Studies using combined multichannel intraluminal impedance with pH monitoring (MII-pH) reveal a positive symptom association with nonacidic reflux and a significant association between non- and weakly acidic reflux and persistent symptoms on PPI therapy [17, 93, 94]. Using multichannel intraluminal impedance (MII)-pH monitoring, Tamhankar et al. [93] showed that PPI therapy decreases the H<sup>+</sup> ion concentration in the refluxed fluid, but does not significantly affect the frequency or duration of reflux events. Kawamura et al. [95] reported on a comparison of GER patterns in 10 healthy volunteers and 10 patients suspected of having reflux-attributed laryngitis. Using a bifurcated MII-pH reflux catheter, the investigators found that gastric reflux with weak acidity (above pH 4) is more common in patients with reflux-attributed laryngitis compared to healthy controls. Oelschlager et al. [96] demonstrated that the majority of reflux episodes into the pharynx are in fact nonacidic. Sharma et al. [17] reported that 70/200 (35%) patients on at least twice daily PPI had a positive symptom index for nonacidic reflux. Finally, Tutuiian et al. [94] also recently reported that reflux episodes extending proximally are significantly associated with symptoms irrespective of the pH of the refluxate.

Patients with signs and symptoms associated with nonacidic and weakly acidic reflux would likely have a negative pH test and would not benefit from PPI therapy. Diagnosis and treatment has focused on the acidity of the refluxate because it was thought that the other components of the refluxate would not be injurious at higher pH. However, it is now known that certain bile acids are injurious at higher pH [97, 98], and recent data supports a role for pepsin in reflux-attributed laryngeal injury

and disease, independent of the pH of the refluxate [99, 100]. Given (1) the multiple reports of refractory reflux-attributed laryngeal symptoms and endoscopic findings on maximal PPI therapy, (2) that studies using MII-pH reveal a positive symptom association with non- and weakly acidic reflux events, and (3) we now know that pepsin and bile acids are injurious at higher pH, the role of acid alone in reflux-attributed signs and symptoms has to be questioned and, subsequently, the efficacy of acid suppression therapy alone for treating reflux.

Anti-reflux fundoplication surgery is one of the few options for patients with persistent reflux-attributed symptoms and endoscopic findings. Unlike medical therapy, which does not stop reflux from occurring but only increases the pH of the refluxate, surgical therapy restores the physiologic separation between the abdomen and thorax and strengthens the LES, thereby preventing refluxate from coming up the esophagus. Iqbal et al. [101] recently reported a study in which 85% of patients who had a fundoplication for extra-esophageal symptoms of reflux had a positive outcome. The majority of these patients had refractory symptoms on maximal acid suppression therapy. Once again, these findings question the role of acid alone in the development of reflux-related pathology and highlight a potential role for the other components of the refluxate.

A role for pepsin in nonacid reflux has been postulated in the recent literature. As already stated, this enzyme is maximally active at pH 2 but can cause tissue damage above this pH, with complete inactivation not occurring until pH 6.5 is reached [21, 22, 102]. While pepsin is inactive at pH 6.5, it remains stable up to pH 8 and thus can be reactivated when the pH is reduced. Pepsin is not irreversibly inactivated until pH 8.0 is reached [1, 22]. Thus, even if pepsin that has been detected in, for example, laryngeal epithelia, is inactive [66, 80] (mean pH of the laryngopharynx is 6.8), it would be stable and thus could reside in an inactive/dormant form in the laryngopharynx but have the potential to become reactivated by a decrease in pH, either triggered by a subsequent acid reflux event that lowers ambient pH or, perhaps, once it is taken up by laryngeal epithelial cells and transported through an intracellular compartment of lower pH [99, 100, 103, 104].

We have proposed mechanisms by which pepsin may cause laryngeal epithelial cell damage at pH 7, and this may occur when refluxate is nonacidic. This may help explain why some patients have refractory symptoms on maximal PPI therapy as well as help explain the reported symptom association with nonacidic reflux events. We have reported mitochondrial and Golgi damage in laryngeal epithelial cells exposed to pepsin at pH 7 [103], and cell toxicity was demonstrated using the MTT cytotoxicity assay. Pepsin at pH 7 was also found to significantly alter the expression levels of multiple genes implicated in stress and toxicity. Of greatest clinical significance, pepsin (0.1 mg/ml, pH 7) was found to induce a pro-inflammatory cytokine gene expression profile in hypopharyngeal FaDu epithelial cells *in vitro* under conditions that were similar to those that contribute to disease in gastroesophageal reflux patients [104]. Collectively, these data suggest a mechanistic link between exposure to pepsin (even in nonacidic refluxate) and cellular changes that lead to laryngopharyngeal inflammatory disease. In this context, the unexpected finding that pepsin is taken up by human laryngeal epithelial cells by receptor-mediated

endocytosis is highly relevant. Pepsin has been previously assumed to cause damage by its proteolytic activity alone, but the discovery that pepsin is taken up by laryngeal epithelial cells by receptor-mediated endocytosis opens the door to a new mechanism for cell damage. It is possible that inactive, but stable, pepsin at pH 7 taken up by laryngeal epithelial cells becomes reactivated once inside the cell in compartments of lower pH, such as late endosomes and the trans-reticular Golgi (TRG) where pepsin's presence has been confirmed [100]. The role of pepsin in nonacid extra-esophageal reflux that can reach other sites of the aerodigestive tract including the lung needs to be investigated, since the refluxate is not likely to be acidic by the time it reaches these proximal structures. The therapeutic potential of receptor antagonists and irreversible inhibitors of peptic activity to prevent pepsin uptake and/or reactivation are currently being studied.

## **Other Clinical Manifestations of EER**

EER has been implicated as a source or cofactor of inflammatory disease of the mucosa of the entire head and neck. Subsites affected by reflux include mucosa of the nose, paranasal sinuses, eustachian tube and middle ear, nasopharynx, oropharynx, hypopharynx, larynx, subglottis, trachea, and lower airway. Connection of EER to specific disease states has been demonstrated in an ever expanding list of conditions of the aerodigestive tract including otitis media, sinusitis, cough, sleep-disordered breathing, laryngitis, laryngospasm, airway stenosis, and lower airway problems such as asthma, chronic obstructive pulmonary disease, interstitial pulmonary fibrosis, and chronic lung transplant rejection. Evidence for these has been based largely on clinical findings that correlate with pH probe studies confirming extra-esophageal reflux or detection of elements of refluxate in the subsite in question. Animal and basic science studies have been used to propose or confirm a mechanism, but in most cases, direct cause and effect in the human condition has yet to be confirmed. Mechanism for disease is typically explained as occurring either by direct contact of refluxate and resulting inflammation of the mucosa or via a vagally mediated neurogenic process as previously discussed.

## **Key Points**

- Reflux is a common source of chronic inflammation in the esophagus and laryngopharynx.
- LPR is different from GERD. Common symptoms of LPR include hoarseness, cough, throat clearing, globus sensation, and dysphagia. GER is more commonly associated with heartburn.
- Intermittent reflux is a physiological process and does not result in injury/disease in the majority of the population, as there are several defense mechanisms present

to protect against its damaging factors. When reflux is in excess and/or there is a breakdown in the defense mechanisms, injury and disease result.

- Extra-esophageal reflux has been implicated in the development of multiple conditions of the aerodigestive tract including Barrett's esophagus, laryngitis, pharyngitis, otitis media, sinusitis, chronic cough, airway stenosis, asthma, and other respiratory diseases. It is also thought to play a significant role in carcinogenesis of the esophagus and laryngopharynx.
- Recent evidence highlights a role for nonacid reflux in injury and disease. Pepsin and bile acids can cause cell damage at high pH and thus in nonacidic reflux. The potential therapeutic role of pepsin inhibitors and receptor antagonists is currently being studied.

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