

ALIMENTARY TRACT

Proton Pump Inhibitors Partially Restore Mucosal Integrity in Patients With Proton Pump Inhibitor–Responsive Esophageal Eosinophilia but Not Eosinophilic Esophagitis



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BACKGROUND & AIMS: Histologic analysis is used to distinguish patients with proton pump inhibitor-responsive eosinophilia (PPI-REE) from those with eosinophilic esophagitis (EoE). It is not clear whether these entities have different etiologies. Exposure to acid reflux can impair the integrity of the esophageal mucosal. We proposed that patients with EoE and PPI-REE might have reflux-induced esophageal mucosal damage that promotes transepithelial flux of allergens. We therefore assessed the integrity of the esophageal mucosal in these patients at baseline and after PPI.

METHODS: We performed a prospective study of 16 patients with suspected EoE and 11 controls. Patients had dysphagia, endoscopic signs of EoE, and esophageal eosinophilia (>15 eosinophils/high-power field [eos/hpf]). All subjects underwent endoscopy at baseline; endoscopy was performed again on patients after 8 weeks of treatment with high-dose esomeprazole. After PPI treatment, patients were diagnosed with EoE (>10 eos/hpf; n = 8) or PPI-REE (≤10 eos/hpf; n = 8). We evaluated the structure (intercellular spaces) and function (electrical tissue impedance, transepithelial electrical resistance, transepithelial molecule flux) of the esophageal mucosal barrier.

RESULTS: Compared with controls, electrical tissue impedance and transepithelial electrical resistance were reduced in patients with EoE ($P < .001$ and $P < .001$, respectively) and PPI-REE ($P = .01$ and $P = .06$, respectively), enabling transepithelial small-molecule flux. PPI therapy partially restored these changes in integrity and inflammation in patients with PPI-REE, but not in those with EoE.

CONCLUSIONS: The integrity of the esophageal mucosa is impaired in patients with EoE and PPI-REE, allowing transepithelial transport of small molecules. PPI therapy partially restores mucosal integrity in patients with PPI-REE, but not in those with EoE. Acid reflux might contribute to transepithelial allergen flux in patients with PPI-REE. Trialregister.nl number: NTR3480.

Keywords: Epithelial Barrier; Pathophysiology; Transepithelial Resistance; Treatment.

See editorial on page 1830; see similar article on page 1824 in this issue of *Clinical Gastroenterology and Hepatology*.

Eosinophilic esophagitis (EoE) is a rapidly emerging disorder clinically characterized by dysphagia and food impaction.¹ The pathophysiology is largely unknown, although genetic and allergic components seem to play a role.² More recently, gastroesophageal reflux disease (GERD) also has been suggested to play a role.^{3–5} A proton pump inhibitor (PPI) trial may

differentiate between EoE and GERD, however, response to PPIs also has been observed in patients with typical

Abbreviations used in this paper: CCL26, eotaxin-3; EoE, eosinophilic esophagitis; eos/hpf, eosinophils/high-power field; ETIS, electrical tissue impedance spectroscopy; FLG, filaggrin; GERD, gastroesophageal reflux disease; IL, interleukin; IQR, interquartile range; mcs/hpf, mast cells/high-power field; PCR, polymerase chain reaction; POSTN, periostin; PPI, proton pump inhibitor; PPI-REE, proton pump inhibitor–responsive eosinophilia; TER, transepithelial electrical resistance.

symptoms and endoscopic and histopathologic signs of EoE; these patients now are considered to have PPI-responsive eosinophilia (PPI-REE).⁴ Little is known about the differences between PPI-REE and EoE.⁶ PPI-REE patients may have GERD instead of EoE, however, they cannot be distinguished from EoE patients by clinical features, endoscopic signs, or histopathologic signs.^{4,7}

In patients with GERD, it has been well documented that acid reflux causes impaired esophageal mucosal integrity, which has been suggested to decrease esophageal intraluminal baseline impedance.^{8,9} Acid suppression with PPIs restores the esophageal mucosal integrity in GERD.¹⁰ In EoE, esophageal baseline impedance values are decreased as well, and histologic studies also have shown dilation of intercellular spaces—a morphologic feature of epithelial permeability changes—in EoE.^{11,12}

We hypothesized that acid-induced impairment of esophageal mucosal integrity facilitates permeation of food allergens, thereby promoting immune activation in patients with PPI-REE and EoE. If this hypothesis is valid, proton pump inhibition will restore the esophageal mucosal integrity and thereby reduce the passage of allergens into the epithelium. The aim of our study was to show an impaired esophageal mucosal integrity in PPI-REE and EoE patients, and to study the effect of acid suppression on the esophageal mucosal integrity.

Methods

Study Subjects

In this prospective study, we included 16 adult patients with suspected EoE (>15 eosinophils/high-power field [eos/hpf], predominant symptoms of dysphagia and/or food impaction, and endoscopic signs of EoE), and 11 healthy controls. Based on peak eosinophil counts after PPI, patients were divided into 2 subgroups: responders (PPI-REE, ≤ 10 eos/hpf) and nonresponders (EoE, >10 eos/hpf) (Figure 1). Patients were recruited consecutively from the outpatient clinic of our hospital. Healthy controls were recruited by advertisement in the hospital and had no dysphagia/reflux symptoms or other gastrointestinal complaints. None of the study subjects had undergone surgery of the digestive tract. Each study subject provided written informed consent and the study protocol was approved by the Medical Ethics Committee of our institution. All authors had access to the study data and reviewed and approved the final manuscript.

Study Protocol

In all study subjects, dietary, anti-inflammatory, and acid-suppressive treatments were discontinued 8 weeks before baseline upper endoscopy. In the 24 hours preceding endoscopy, smoking and alcohol intake were not allowed. In patients, endoscopy was repeated after 8

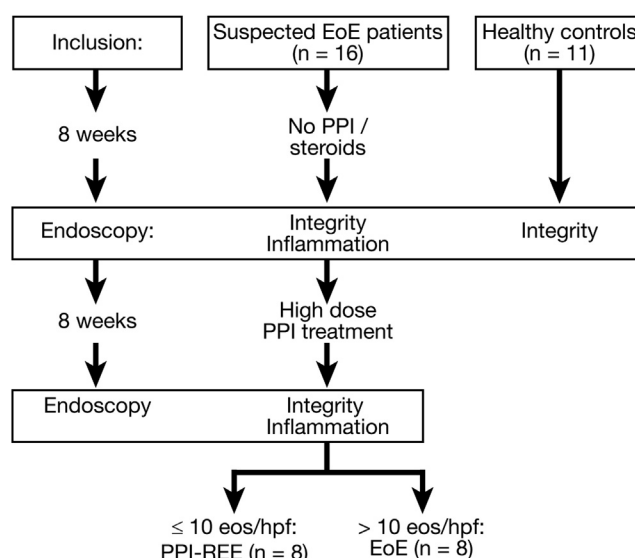


Figure 1. Study protocol and patient subgroup selection. The esophageal mucosal integrity of patients and controls was measured at baseline. In patients, the integrity and inflammation were measured after PPI treatment. Based on the eosinophil count after PPI treatment, patients were categorized as PPI-REE (≤ 10 eos/hpf) or EoE (>10 eos/hpf).

weeks of 40 mg esomeprazole twice-daily treatment, and the frequency and severity of dysphagia for liquids and solids was assessed using a 6-grade Likert scale, in which 0 represents no dysphagia and 5 represents daily/severe dysphagia, analogous to the reflux disease questionnaire.

Upper Endoscopy

After routine inspection of the duodenum and stomach, pictures were taken of the esophagus for assessment of endoscopic signs of EoE. After this, 5 electrical tissue impedance spectroscopy (ETIS) measurements were performed in the distal esophagus 5 cm proximal to the Z-line. At the same level, 4 biopsy specimens were obtained with a large biopsy forceps (diameter, 3.7 mm) for the assessment of functional mucosal integrity in Ussing chambers. In addition, 2 large biopsy specimens were taken to evaluate dilation of intercellular spaces by transmission electron microscopy. For histopathologic analysis in patients, 6 biopsy specimens were taken, and 1 biopsy specimen was taken for gene expression profiling.

To assess endoscopic signs of EoE (white exudates, linear furrows, concentric rings, solitary ring, crepe paper mucosa, pallor, and narrow-caliber esophagus), a physician blinded to the patient's status scored the endoscopic pictures.

Esophageal Mucosal Integrity Assessment In Vivo

As a functional measure of esophageal mucosal integrity, we performed ETIS measurements to measure the

extracellular esophageal impedance during endoscopy, as previously described.¹³ The extracellular impedance was correlated with structural and functional in vitro parameters of esophageal mucosal integrity; ETIS is therefore a measure of esophageal mucosal integrity in vivo.¹³

Esophageal Mucosal Integrity Assessment In Vitro

Esophageal mucosal integrity was assessed according to previously described methods.¹³ In short, 4 esophageal biopsy specimens were mounted in Ussing chambers immediately after endoscopy. Electrodes were used to measure the transepithelial electrical resistance (TER). Simultaneously, the transepithelial flux of fluorescent molecules of 2 different sizes (fluorescein, 332 daltons; rhodamine, 40,000 daltons; size similar to common food allergens) was measured. After 15 minutes of acclimatization in Meyler buffer, we sampled the serosal bath and subsequently replaced the luminal buffer with a modified Meyler buffer containing these fluorescent molecules at a concentration of 0.5 mg/mL. We sampled the serosal bath every 15 minutes during 1 hour. A fluorescence plate reader (BioTek Synergy; BioTek, Winooski, VT) measured the concentration of fluorescein and rhodamine molecules using excitation wavelengths of 485 and 530 nm and emission wavelengths of 528 and 590 nm, respectively. Rhodamine signal strength was corrected for the presence of fluorescein.

Transmission Electron Microscopy

As a structural marker of esophageal mucosal integrity related to esophageal permeability changes,^{8,10} the space between individual esophageal epithelial cells was measured, as previously described.¹³ The laboratory technician was blinded to the status of the biopsy and selected 10 random photographs of each biopsy at the basal prickly layer (magnification, 4600 $\times$). Image processing and analysis was performed using Qwin (Leica Microsystems, Wetzlar, Germany).

Histopathologic Analysis

For histopathologic analysis, 2 biopsy specimens were taken at the distal esophagus, midesophagus (5 cm and 10–15 cm above the gastroesophageal junction), and proximal esophagus (5 cm below the upper esophageal sphincter). Specimens were stained with H&E and tryptase to determine the eosinophil and mast cell counts. An experienced gastrointestinal pathologist blinded to biopsy location and the patient's treatment status analyzed the specimens in random order, using an Olympus BX41 microscope (Olympus Europe, Hamburg, Germany). In each biopsy specimen, the area of greatest eosinophil density was detected with a low-power view. By using a

magnification of 400 $\times$ (1 hpf), the peak eosinophil count was determined. Mast cells were counted identically.

Furthermore, the presence of eosinophilic microabscesses (defined as clusters of ≥ 4 eosinophils) were analyzed at high-power, and basal hyperplasia and spongiosis were analyzed at low-power (for both: 0 = absent, 1 = extending to lower third of total epithelial thickness [mild], 2 = extending to middle third [moderate], 3 = extending to upper third [severe]), according to the literature.¹¹

Quantitative Real-Time Polymerase Chain Reaction

During endoscopy at baseline and after PPI, 1 mucosal biopsy specimen from each patient was collected and immersed in RNA stabilization reagent (RNAlater; Qiagen, Hilden, Germany). Samples were stored overnight at 4°C and subsequently were stored at -80°C until analysis. Biopsy specimens were homogenized and total RNA was extracted using the RNeasy Micro Kit (Qiagen) according to the manufacturer's recommendations. The RNA concentration was assessed using the Nanodrop Spectrophotometer (Nanodrop Technologies, Wilmington, DE). Complementary DNA was synthesized using a reverse-transcriptase reaction performed according to the MBI Fermentas complementary DNA synthesis kit (Fermentas, Vilnius, Lithuania), using both the Oligo(dT)18 and the D(N)6 primers. Quantitative real-time polymerase chain reaction (PCR) was performed on the LightCycler 480 (Roche Diagnostic, Almere, The Netherlands) using SYBR Green PCR Master Mix (Roche Diagnostic) and primers from Invitrogen (Life Technologies Corporation, Carlsbad, CA) (Supplementary Table 1). For quantitative real-time PCR, samples were normalized for the mean of the 3 most stable housekeeping genes (*cyclophilin*, *glyceraldehyde-3-phosphate dehydrogenase*, and β -actin) as determined by analysis with geNorm method software (available at: <http://medgen.ugent.be/~jvdesomp/genorm/>). Transcript levels of interleukin (*IL*)5, *IL*13, eotaxin-3 (*CCL26*), periostin (*POSTN*), filaggrin (*FLG*), and thymic stromal lymphopoietin were determined in duplicate.

Statistical Analysis

Continuous data were expressed as medians (interquartile range [IQR]). Differences between 2 or more groups were calculated with the Kruskal-Wallis test, with Bonferroni post hoc correction for multiple testing. Patient groups were compared using the Mann-Whitney *U* test or the Wilcoxon signed rank test where appropriate. Proportions were compared using the chi-square test, the Fisher exact test, or the McNemar test where appropriate. Correlations were calculated using the Spearman correlation coefficient. We considered a *P* value less than .05 to be significant.

Results

Patients vs Controls

Subject characteristics. We consecutively included 16 patients (13 men) with suspected EoE, and 11 controls (7 men). No patients were excluded or dropped out after enrolment in the study. The median age at first endoscopy was 42 years (IQR, 32–46 y) for patients and 35 years (IQR, 29–53 y) for controls ($P = .6$). None of the controls had abnormalities on endoscopy. Based on peak eosinophil counts after PPI, 8 patients (6 men) were classified as PPI-REE (range, 0–10 eos/hpf), and 8 patients (7 men) were classified as EoE patients (range, 19–100 eos/hpf).

Esophageal mucosal integrity and intercellular spaces. The distributions of the extracellular impedance ($P < .001$), TER ($P < .001$), fluorescein flux ($P = .001$), and intercellular spaces ($P = .003$) were significantly different between PPI-REE and EoE patients and healthy controls. Post hoc analysis showed that the extracellular impedance was significantly lower in EoE (2014 $\Omega\cdot\text{m}$; 1288–3963 $\Omega\cdot\text{m}$; $P < .001$) and PPI-REE patients (3128 $\Omega\cdot\text{m}$; 1869–5213 $\Omega\cdot\text{m}$; $P = .01$) compared with controls (7707 $\Omega\cdot\text{m}$; 6146–10,488 $\Omega\cdot\text{m}$; (Figure 2A). TER values also were lower in EoE (31.3 $\Omega\cdot\text{cm}^2$; 25.4–41.8 $\Omega\cdot\text{cm}^2$; $P < .001$) and borderline lower in PPI-REE patients (42.7 $\Omega\cdot\text{cm}^2$; 34.1–62.4 $\Omega\cdot\text{cm}^2$; $P = .06$) than in controls (116.7 $\Omega\cdot\text{cm}^2$; 90.3–126.4 $\Omega\cdot\text{cm}^2$) (Figure 2B). Furthermore, compared with controls (345 nmol/cm²/h; 0–1451 nmol/cm²/h), the transepithelial flux of fluorescein molecules in the Ussing chambers was significantly increased in EoE patients (2974 nmol/cm²/h; 2680–3362 nmol/cm²/h; $P <$

.001), but not in PPI-REE patients (1640 nmol/cm²/h; 1–2424 nmol/cm²/h; $P = .8$) (Figure 2C).

At baseline, intercellular spaces were evaluated in all EoE patients, in 7 PPI-REE patients, and in 6 controls. Samples of 1 PPI-REE patient and 5 control subjects unfortunately were lost as a result of a technical problem during sample processing. The intercellular space was significantly larger in EoE (0.34; 0.32–0.45; $P = .008$) and PPI-REE patients (0.37; 0.33–0.42; $P = .007$) than in controls (0.14; 0.11–0.23) (Figure 2D).

Effect of Proton Pump Inhibitor

Esophageal mucosal integrity and intercellular spaces. In PPI-REE patients (Figure 3), the extracellular impedance increased significantly from 3128 $\Omega\cdot\text{m}$ (1869–5213 $\Omega\cdot\text{m}$) to 6848 $\Omega\cdot\text{m}$ (5761–9363 $\Omega\cdot\text{m}$) ($P = .01$) after PPI, and was no longer different from healthy control values ($P = .6$). The TER increased from 42.7 $\Omega\cdot\text{cm}^2$ (34.1–62.4 $\Omega\cdot\text{cm}^2$) to 100.0 $\Omega\cdot\text{cm}^2$ (68.8–121.9 $\Omega\cdot\text{cm}^2$; $P = .004$), and was not different from healthy control values ($P = 1.0$). The transepithelial flux of the small fluorescein molecules decreased from 1640 nmol/cm²/h (1–2424 nmol/cm²/h) to 255 nmol/cm²/h (39–628 nmol/cm²/h), although not significantly ($P = .06$), and was not different from healthy control values ($P = 1.0$). Moreover, the flux of the larger, food allergen-sized rhodamine molecules decreased significantly from 22 nmol/cm²/h (0–38 nmol/cm²/h) to 0 nmol/cm²/h (0–0 nmol/cm²/h; $P = .046$). The intercellular space remained unchanged, being 0.37 (0.33–0.42) at baseline and 0.33 (0.27–0.38) after PPI ($P = .1$).

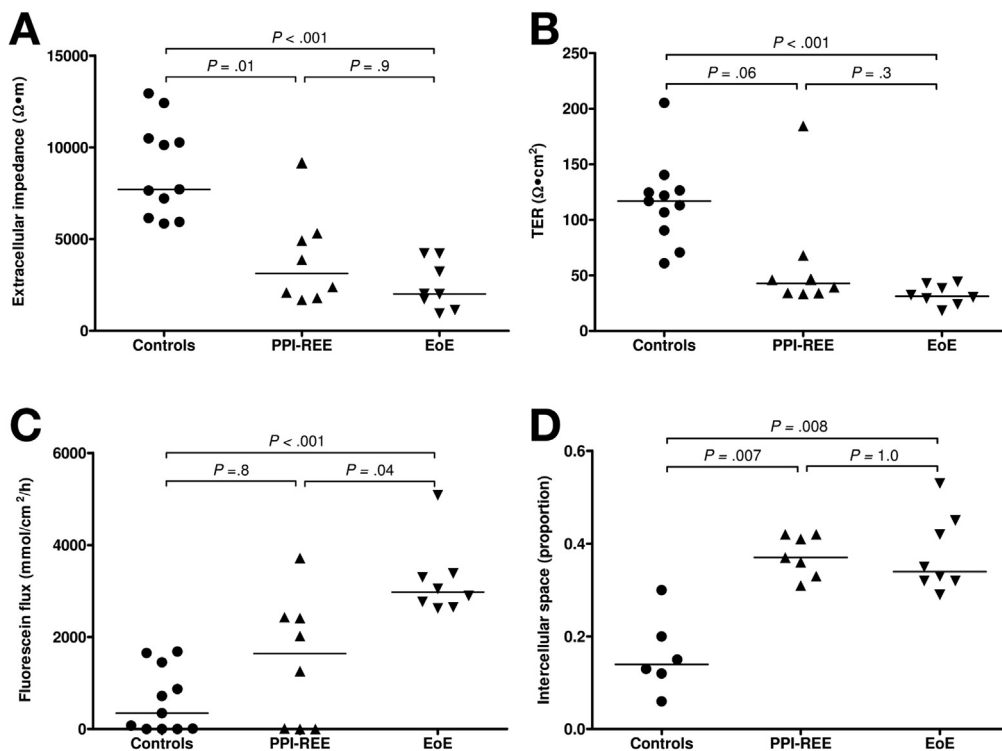


Figure 2. (A) Extracellular impedance, (B) TER, (C) fluorescein flux, and (D) intercellular space show impaired mucosal integrity in PPI-REE patients and EoE patients compared with controls. Black bars indicate medians.

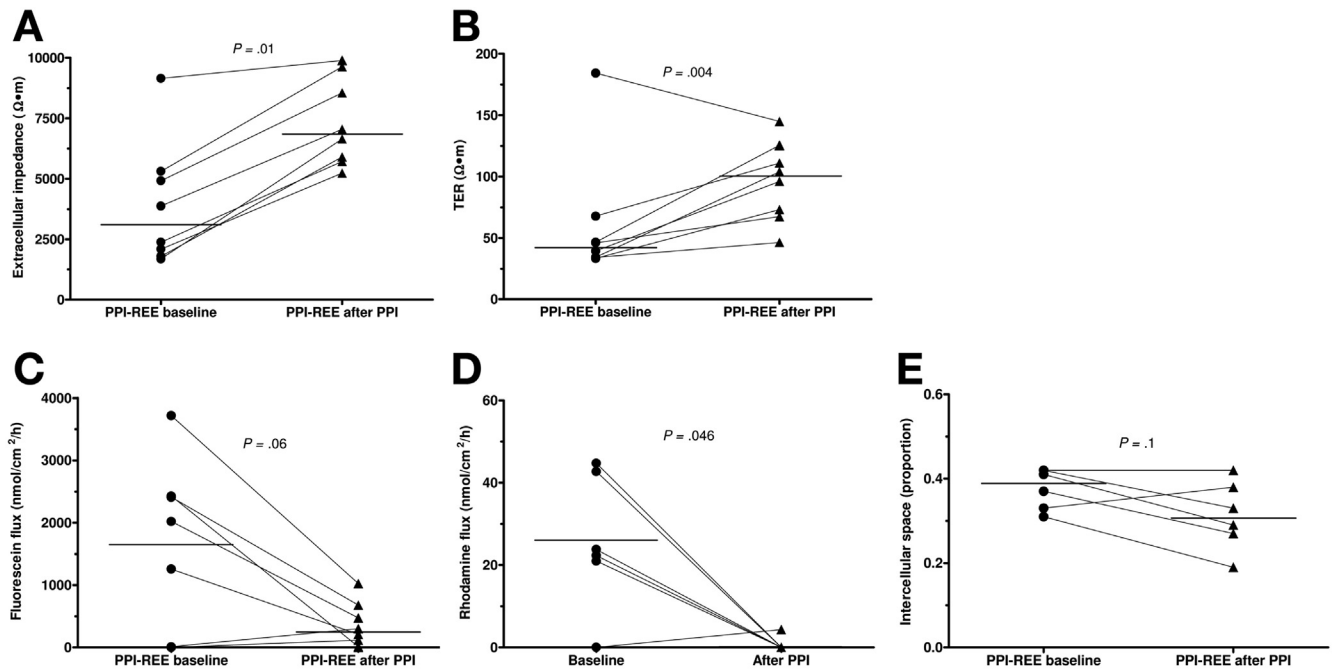


Figure 3. (A) Extracellular impedance, (B) TER, (C) fluorescein flux, (D) rhodamine flux, and (E) intercellular space in PPI-REE patients after PPI. Black bars indicate medians.

In EoE patients (Figure 4), the extracellular impedance increased significantly from 2014 $\Omega\bullet m$ (1288–3963 $\Omega\bullet m$) to 3404 $\Omega\bullet m$ (2810–6579 $\Omega\bullet m$; $P = .01$) after PPI, however, it was still significantly lower than in healthy controls ($P = .04$). The TER (from 31.3 $\Omega\bullet m$; 25.4–41.8 to 32.4 $\Omega\bullet m$; 27.1–56.5 $\Omega\bullet m$ after PPI; $P = .9$), the transepithelial flux of small fluorescein molecules (from 2974 nmol/cm²/h; 2680–3362 nmol/cm²/h to 2502 nmol/cm²/h; 1404–3251 nmol/cm²/h after PPI; $P = .1$),

and larger rhodamine molecules (from 20 nmol/cm²/h; 7–38 nmol/cm²/h to 9 nmol/cm²/h; 5–28 nmol/cm²/h after PPI; $P = .3$), and the intercellular space (from 0.34; 0.32–0.45 to 0.35; 0.33–0.42 after PPI; $P = .4$) were unaffected by PPI. After PPI, the TER was significantly lower in EoE patients compared with healthy controls ($P = .001$) and compared with PPI-REE patients ($P = .03$). In line with this, the flux of small fluorescein molecules ($P = .004$ vs controls, and $P = .003$ vs PPI-REE

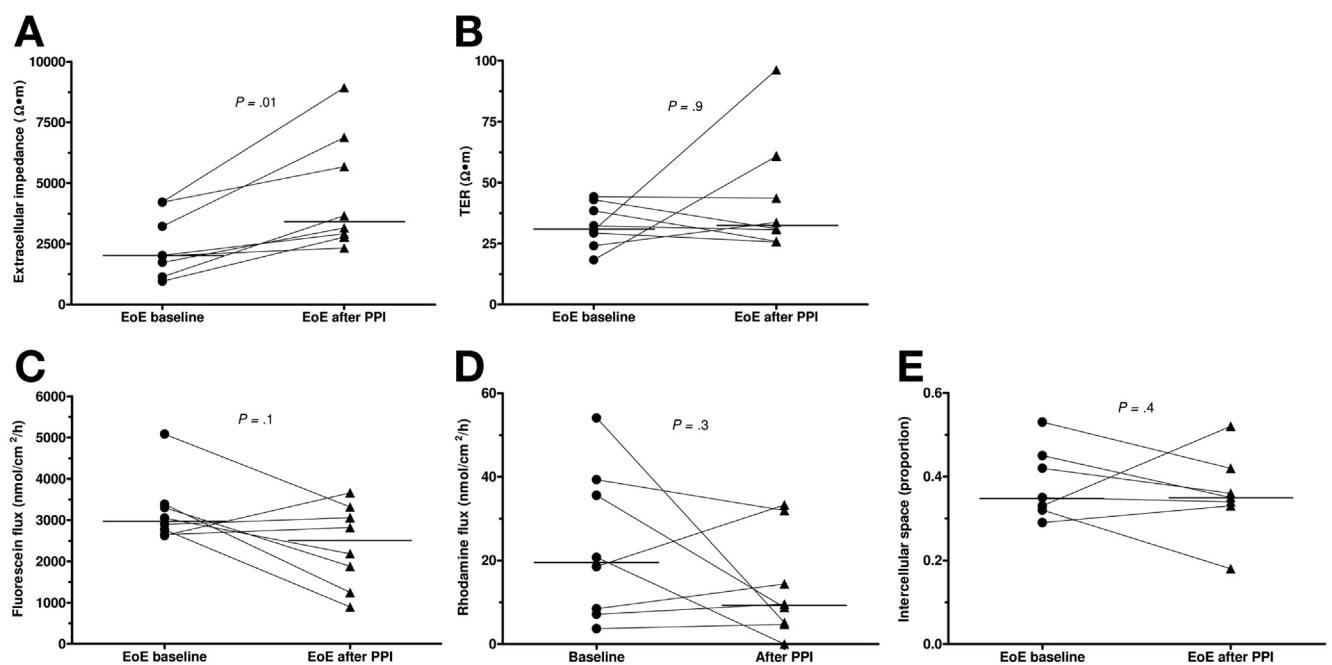


Figure 4. (A) Extracellular impedance, (B) TER, (C) fluorescein flux, (D) rhodamine flux, and (E) intercellular space in EoE patients after PPI. Black bars indicate medians.

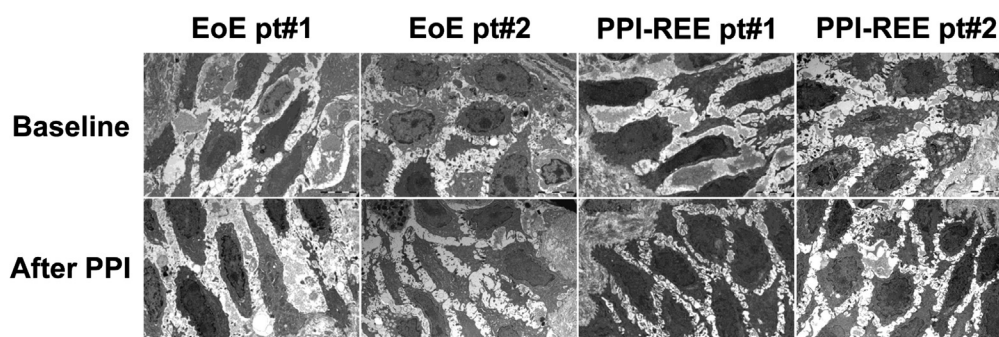


Figure 5. Transmission electron microscopy images of the basal prickles layer of esophageal mucosal biopsy specimens showing dilated intercellular spaces. Intercellular spaces were not different between PPI-REE and EoE patients at baseline and after PPI. Nevertheless, in some PPI-REE patients (eg, PPI-REE patient 1), the intercellular spaces were reduced after PPI. Scale bars: 5 μ m. pt#, patient number.

patients) and the larger rhodamine molecules ($P = .002$ vs PPI-REE patients) still was high in EoE patients after PPI. Intercellular spaces were not different between PPI-REE patients and EoE patients at baseline and after PPI (both $P = 1.0$) (Figure 5).

Histopathology. PPI reduced peak eosinophil counts in the distal esophagus, midesophagus, and proximal esophagus in PPI-REE patients, whereas in EoE patients no effect of PPI on eosinophil counts was observed (Supplementary Table 2). No differences were found in the baseline eosinophil counts of the distal esophagus, midesophagus, and proximal esophagus between PPI-REE and EoE patients (distal esophagus, $P = .1$; midesophagus, $P = .4$; proximal esophagus, $P = .1$). However, after PPI, eosinophil counts were significantly lower at all esophageal levels in PPI-REE patients than in EoE patients (distal esophagus, $P = .001$; midesophagus, $P = .002$; proximal esophagus, $P < .001$).

In PPI-REE patients, the peak mast cell count in the distal esophagus also was decreased after PPI, although significance was not reached (from 19.5 mast cells per high-power field [mcs/hpf]; 10.0–36.5 mcs/hpf to 10.5 mcs/hpf; 4.0–20.8 mcs/hpf; $P = .07$). Peak mast cell counts were not affected by PPI in the midesophagus (from 8.5 mcs/hpf; 5.0–28.8 mcs/hpf to 6.5 mcs/hpf; 6.0–11.3 mcs/hpf; $P = .2$) and proximal esophagus (from 9.5 mcs/hpf; 3.3–22.3 mcs/hpf to 8.0 mcs/hpf; 4.8–13.5 mcs/hpf; $P = .4$) of PPI-REE patients. In EoE patients, PPIs did not affect peak mast cell counts in the distal esophagus, midesophagus, and proximal esophagus (distal from 32.5 mcs/hpf; 12.0–48.8 mcs/hpf to 23.0 mcs/hpf; 11.5–29.3 mcs/hpf, $P = .3$; midesophagus from 16.0 mcs/hpf; 4.5–24.3 mcs/hpf to 15.0 mcs/hpf; 2.5–44.5 mcs/hpf; $P = .7$; proximal from 21.5 mcs/hpf; 14.3–36.3 mcs/hpf to 23.5 mcs/hpf; 7.8–32.5 mcs/hpf; $P = .6$).

At baseline, eosinophilic microabscesses were present in 4 PPI-REE patients and in all EoE patients ($P = .08$). After PPI, eosinophilic microabscesses were not seen in PPI-REE patients, whereas they were found in 6 EoE patients ($P = .007$). In more EoE patients than PPI-REE patients, moderate to severe basal hyperplasia was observed at baseline (8 vs 5; $P = .2$) and after PPI (6 vs 1;

$P = .04$). Basal hyperplasia was not altered significantly by PPI treatment in either PPI-REE patients or EoE patients ($P = .1$ and $P = .5$, respectively). Spongiosis was moderate to severe in more EoE patients than PPI-REE patients at baseline (7 vs 3; $P = .1$) and after PPI (7 vs 1; $P = .01$). The degree of spongiosis remained unchanged in PPI-REE patients ($P = .5$) and EoE patients ($P = 1.0$) after PPI.

Cytokine expression. Analyses of the biopsy gene expression patterns (Supplementary Table 3) showed no differences in baseline allergic cytokine expression between PPI-REE and EoE patients. In PPI-REE patients, the median expression of *IL5*, *CCL26*, and *POSTN* decreased after PPI. PPI treatment also caused an increase in the expression of *FLG* transcript. Esophageal expression levels of *IL13* and thymic stromal lymphopoietin were unaffected by PPI. In EoE patients, *FLG* expression increased after PPI, whereas expression of inflammatory genes was not affected by PPI. After PPI, PPI-REE patients expressed significantly lower levels of *CCL26* and *POSTN* than EoE patients.

Symptoms. In PPI-REE patients, the frequency of dysphagia decreased from 1.5 (1.0–8.5) to 0.0 (0.0–1.0) ($P = .03$) after PPI, and the severity decreased from 3.0 (1.0–6.5) to 0.0 (0.0–1.8) ($P = .03$). In contrast, in EoE patients, PPI treatment did not reduce frequency (1.0; 0.0–8.5 vs 0.0; 0.0–2.0; $P = .1$) or severity of dysphagia (2.5; 0.0–5.8 vs 0.0; 0.0–3.5; $P = .2$). Despite this difference, dysphagia frequency and severity scores were not significantly different between PPI-REE patients and EoE patients at baseline and after PPI.

Endoscopic signs. At baseline, pallor was seen in all EoE and PPI-REE patients, esophageal rings in 7 EoE and 7 PPI-REE patients, linear furrows in 7 EoE and 6 PPI-REE patients, crêpe-paper mucosa in 7 EoE and 4 PPI-REE patients, white exudates in 5 EoE and 6 PPI-REE patients, narrow-caliber esophagus in 3 EoE and 2 PPI-REE patients, and a solitary ring in 1 EoE patient. At baseline, none of these signs were significantly different between PPI-REE and EoE patients.

In PPI-REE patients, the number of endoscopic signs decreased after PPI from 4.0 (3.3–4.8) to 2.5 (0.5–3.8)

($P = .02$). In EoE patients, the number of endoscopic signs was not affected (5.0; 3.3–5.8 at baseline and 4.0; 3.0–5.0 after PPI; $P = .2$). At baseline, the number of endoscopic signs did not differ between PPI-REE patients and EoE patients ($P = .3$), however, after PPI, PPI-REE patients had significantly fewer endoscopic signs than EoE patients ($P = .04$). Linear furrows were found in more EoE (7 patients) than PPI-REE (2 patients) patients after PPI (2 patients) (Figure 6).

Histologic Response to Treatment Correlates With Esophageal Integrity

Peak eosinophil counts after PPI therapy correlated with the extracellular impedance ($r = -0.54$; $P = .03$), the TER ($r = -0.68$; $P = .004$) the flux of fluorescein molecules ($r = 0.79$; $P < .001$), and the flux of rhodamine molecules ($r = 0.736$; $P = .001$). Accordingly, peak mast cell counts after PPI therapy correlated with the extracellular impedance ($r = -0.82$; $P < .001$), the flux of fluorescein molecules ($r = 0.61$; $P = .01$), and the flux of rhodamine molecules ($r = 0.62$; $P = .01$). In vivo and in vitro esophageal mucosal integrity measurements thus were correlated moderately to strongly with esophageal inflammation. Furthermore, the expression of *FLG* correlated with the TER ($r = 0.78$; $P < .001$), the flux of fluorescein molecules ($r = -0.75$; $P < .001$), and rhodamine molecules ($r = -0.58$; $P = .02$).

Discussion

This study investigated the esophageal mucosal integrity in patients with PPI-REE and EoE. By using structural and functional measurements we found that the esophageal mucosal integrity was impaired, to a similar extent, in patients with PPI-REE and EoE. Furthermore, acid suppression with PPIs partially restored mucosal integrity parameters and decreased inflammation in patients with PPI-REE, but not in patients with EoE.

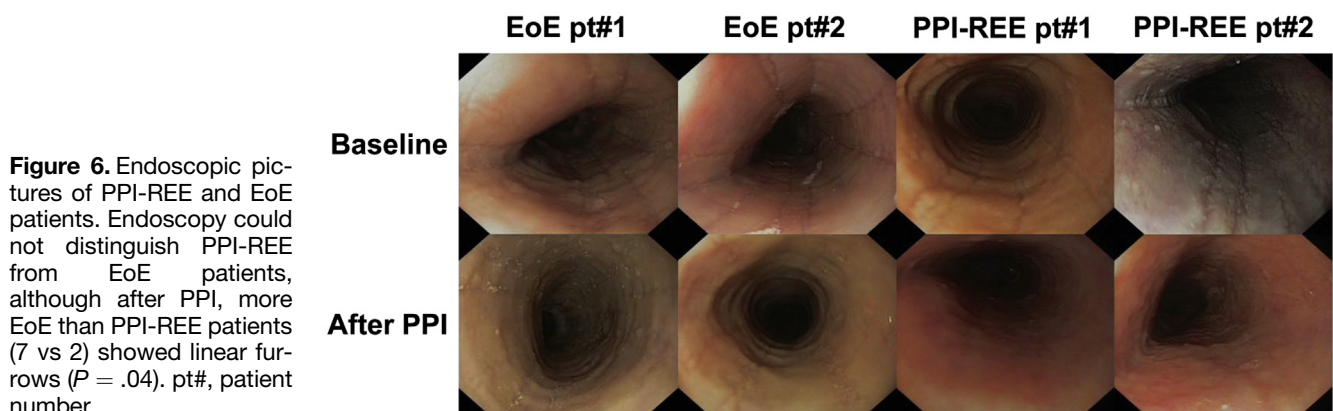
In this study, we showed passage of molecules that were 40,000 daltons through the mucosa in EoE and PPI-REE patients. This size is similar to the size of most plant

and animal food allergens to which EoE patients are sensitized.^{14,15} Our observations suggest that increased permeability of the esophageal mucosa could play an important role in the presentation of allergens to the immune system in EoE and PPI-REE.

Of importance for this study is the distinction between EoE and PPI-REE. Current guideline recommendations state that the diagnosis of EoE should be reserved for patients with symptoms related to esophageal dysfunction, with esophageal eosinophilia, who have an inadequate response to PPIs.⁵ Patients with suspected EoE who do respond to PPIs are diagnosed with PPI-REE, a condition that is considered distinct from EoE.⁴ In concordance with EoE guidelines, a threshold of 15 eos/hpf after PPI is used to distinguish both entities.^{4,5} In our study, we set the threshold at 10 eos/hpf after PPI, although no patients were in the range of 10 to 15 eos/hpf.

It is unknown why PPI-REE patients do and EoE patients do not respond to PPI.⁶ We found that allergic inflammatory cytokines were expressed to the same extent in EoE and PPI-REE patients, suggesting that these entities reflect one and the same disease. Symptoms, endoscopic signs, peak eosinophil counts, and mast cell counts were not different between EoE and PPI-REE patients, and the esophageal mucosal integrity was impaired to a similar extent. It has been suggested that patients with PPI-REE have a less severe variant of EoE. In support of this, more EoE patients than PPI-REE patients had eosinophilic microabscesses, severe basal hyperplasia, and severe spongiosis at baseline in our study. However, as stated, no differences were found in clinical characteristics. The different response to PPI between EoE and PPI-REE patients thus might be explained by other factors than disease severity.

Recently, it also has been suggested that gastro-esophageal reflux contributes to PPI-REE by causing esophageal integrity changes and that this is why PPI-REE patients respond to PPI.⁶ Indeed, in our study, we showed that in PPI-REE patients the esophageal mucosal integrity was impaired and that the integrity partially was restored with PPI. The restored integrity may provide an effective barrier against the permeation of allergens and subsequently may block allergen exposure to



antigen-presenting cells, thereby prohibiting immune activation. The observed effect of acid suppression with high-dose PPIs on the mucosal integrity in PPI-REE patients supports the hypothesis that acid reflux contributes to the impaired epithelial barrier function in PPI-REE. Alternatively, it also is possible that restoration of the mucosal integrity is the result of a direct anti-inflammatory effect of PPIs on the esophageal epithelium. Unfortunately, pretreatment predictive factors for PPI response have not been identified yet, either in *in vitro* studies or in pH-monitoring studies.

An alternative cause of impaired mucosal integrity could be that the intrinsic inflammatory cytokines and cytotoxic eosinophil and mast cell secretory products that are present in the esophagus of EoE and PPI-REE patients impair the mucosal integrity, similar to mechanisms in asthma and atopic dermatitis.^{16,17} The loss of desmoglein-1, an intercellular adhesion molecule that is altered in various skin disorders, may potentiate this allergic inflammation through the induction of proinflammatory mediators such as POSTN.¹⁸ The observation that EoE patients did not respond to a PPI suggests that gastroesophageal reflux does not play a role in EoE patients, and favors this second explanation for the impaired mucosal integrity in EoE. Furthermore, the strongly decreased expression of *FLG*, previously described in EoE, increased after PPI in PPI-REE but not in EoE patients.¹⁹ The finding that *FLG* expression also correlated inversely with the permeability to allergen-sized molecules suggests that down-regulation of *FLG* also may contribute to the observed epithelial barrier dysfunction.

In conclusion, the esophageal mucosal integrity is severely impaired in patients with PPI-REE and in patients with EoE. This finding could be of pathophysiological importance because increased permeability may facilitate transepithelial food allergen flux. Furthermore, the finding that histologic response correlates with improved esophageal barrier integrity suggests that barrier integrity is a potential therapeutic target. The observation that PPI partially improves the esophageal mucosal integrity in PPI-REE patients but not in EoE patients may indicate a role for acid reflux in PPI-REE but not in EoE.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at <http://dx.doi.org/10.1016/j.cgh.2014.02.037>.

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Conflicts of interest

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Supplementary Table 1. Gene Function and Gene-Specific Primers for Real-Time PCR

Gene	Primer	Sequence	Protein encoded	Protein role in EoE ²
<i>IL5</i>	Forward	5'-cgaactctgctgatagccaa-3'	IL5	Attracts eosinophils
	Reverse	5'-cagtacccccttgcacagtt-3'		Regulates response of eosinophils to eotaxin-3
<i>IL13</i>	Forward	5'-ggtcaacatcacccagaacc-3'	IL13	Induces eotaxin-3 production by epithelial cells
	Reverse	5'-gattccagggtgcacagta-3'		Down-regulates filaggrin, up-regulates periostin expression
<i>CCL26</i>	Forward	5'-aactccgaacaattgtactcagctg-3'	Eotaxin-3	Attracts and activates eosinophils
	Reverse	5'-gtaactctgggaggaaacaccctctcc-3'		
<i>POSTN</i>	Forward	5'-ttcaacagattttgggcacc-3'	Periostin	Involved in eosinophil accumulation and cell adhesion
<i>FLG</i>	Reverse	5'-cagcctttcattccttccat-3'	Filaggrin	Induced in remodeling Important for epithelial barrier function
	Forward	5'-accatcttgatctgcttg-3'		
	Reverse	5'-ctgtgtgacgagtgccctgat-3'		
<i>TSLP</i>	Forward	5'-tcgccatgaaaactaaggct-3'	TSLP	Induces T-helper type 2-inflammation and allergic diseases
	Reverse	5'-tctcctcttcttcattgctg-3'		
Housekeeping gene				
<i>Cyclophilin</i>	Forward	5'-gccgaggaaaaccgtgtact-3'		
	Reverse	5'-acttcttggtatccaggccc-3'		
<i>GAPDH</i>	Forward	5'-gagtcaacggatttggtcgt-3'		
	Reverse	5'-ttgattttggagggatctcg-3'		
<i>β-actin</i>	Forward	5'-gtcagaaggattcctatgtgg-3'		
	Reverse	5'-gctcattgccaatggtgatg-3'		

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TSLP, thymic stromal lymphopoietin.

Supplementary Table 2. Eosinophil Counts in PPI-REE Patients and EoE Patients

Patient group	Location	Peak eosinophil count, eos/hpf		P value
		Baseline	After PPI	
PPI-REE, median (IQR)	Proximal	17.5 (0.5–71.3)	1.0 (0.3–3.0)	.03
	Mid	31.0 (1.0–80.0)	1.0 (0.0–1.8)	.03
	Distal	30.0 (3.0–68.8)	1.0 (0.0–1.8)	.04
EoE, median (IQR)	Proximal	47.5 (25.8–107.5)	36.0 (6.8–57.5)	.2
	Mid	60.0 (42.5–70.0)	52.5 (35.8–68.8)	.4
	Distal	55.0 (41.3–92.5)	52.5 (23.3–83.8)	.4

Supplementary Table 3. Gene Expression at Baseline and After PPI in PPI-REE Patients and EoE Patients

Patient group	Gene	Baseline	After PPI	P value
PPI-REE, median (IQR)	<i>IL5</i>	1.0×10^{-2} (6.6×10^{-3} – 1.4×10^{-2})	6.4×10^{-3} (0.0×10^0 – 1.5×10^{-2})	.03
	<i>IL13</i>	2.5×10^{-3} (1.2×10^{-3} – 3.8×10^{-3})	6.3×10^{-4} (0.0×10^0 – 3.4×10^{-3})	.07
	<i>CCL26</i>	1.0×10^2 (5.5×10^1 – 1.8×10^2)	1.2×10^2 (2.8×10^1 – 1.8×10^2)	.03
	<i>POSTN</i>	3.8×10^{-1} (2.8×10^{-1} – 2.3×10^0)	4.8×10^{-1} (8.6×10^{-2} – 1.1×10^0)	.04
	<i>FLG</i>	1.1×10^{-2} (4.8×10^{-3} – 3.8×10^{-2})	1.1×10^{-1} (2.7×10^{-2} – 1.9×10^{-1})	.04
	<i>TSLP</i>	6.1×10^{-2} (4.9×10^{-2} – 1.3×10^{-1})	6.8×10^{-2} (3.3×10^{-2} – 8.3×10^{-2})	.4
EoE, median (IQR)	<i>IL5</i>	5.3×10^{-3} (6.4×10^{-4} – 2.1×10^{-2})	0.0×10^0 (0.0×10^0 – 8.9×10^{-4})	.5
	<i>IL13</i>	3.7×10^{-4} (0.0×10^0 – 2.1×10^{-3})	0.0×10^0 (0.0×10^0 – 7.7×10^{-4})	.2
	<i>CCL26</i>	1.2×10^2 (1.0×10^1 – 2.9×10^2)	1.0×10^1 (5.4×10^0 – 2.2×10^1)	1.0
	<i>POSTN</i>	2.8×10^{-1} (2.3×10^{-2} – 1.2×10^0)	6.2×10^{-2} (7.5×10^{-3} – 1.4×10^{-1})	.3
	<i>FLG</i>	2.1×10^{-2} (1.6×10^{-2} – 3.0×10^{-1})	4.6×10^{-1} (1.1×10^{-1} – 9.3×10^{-1})	.02
	<i>TSLP</i>	6.4×10^{-2} (4.2×10^{-2} – 8.3×10^{-2})	9.0×10^{-2} (3.6×10^{-2} – 1.2×10^{-1})	.8

NOTE. Baseline cytokine expression levels were not significantly different between PPI-REE and EoE patients. Compared with EoE patients, PPI-REE patients had significantly lower levels of *CCL26* ($P = .01$) and *POSTN* ($P = .03$) after PPI.

TSLP, thymic stromal lymphopoietin.