

# Dietary therapy can reverse esophageal subepithelial fibrosis in patients with eosinophilic esophagitis: a historical cohort

J. A. Lieberman<sup>1</sup>, R. A. Morotti<sup>2</sup>, G. N. Konstantinou<sup>1,3</sup>, O. Yershov<sup>4</sup> & M. Chehade<sup>1,4</sup>

<sup>1</sup>Division of Allergy and Immunology, Department of Pediatrics, Mount Sinai School of Medicine, New York, NY; <sup>2</sup>Department of Pathology, Mount Sinai School of Medicine, New York, NY, USA; <sup>3</sup>Department of Allergy, General Military Training Hospital, Thessaloniki, Greece;

<sup>4</sup>Mount Sinai Center for Eosinophilic Disorders, Jaffe Food Allergy Institute, Mount Sinai School of Medicine, New York, NY, USA

**To cite this article:** Lieberman JA, Morotti RA, Konstantinou GN, Yershov O, Chehade M. Dietary therapy can reverse esophageal subepithelial fibrosis in patients with eosinophilic esophagitis: a historical cohort. *Allergy* 2012; DOI: 10.1111/j.1398-9995.2012.02881.x.

## Keywords

corticosteroids; diet; eosinophilic esophagitis; fibrosis; remodeling.

## Correspondence

Mirna Chehade, Mount Sinai School of Medicine, One Gustave L. Levy Place, Box 1198, New York, NY 10029, USA.

Tel.: 212-241-0830

Fax: 212-426-1902

E-mail: mirna.chehade@mssm.edu

Accepted for publication 30 June 2012

DOI:10.1111/j.1398-9995.2012.02881.x

Edited by: Hans-Uwe Simon

## Abstract

**Background:** Fibrosis of the esophageal lamina propria is a known complication of eosinophilic esophagitis (EoE). To date, therapy with topical corticosteroids has been shown to reverse esophageal fibrosis in some patients; however, there is little evidence to suggest that dietary therapy can also reverse it. Our aim was to examine whether dietary therapy alone can reverse esophageal fibrosis in children with EoE.

**Methods:** We performed a historical cohort study based on children with EoE who had esophageal fibrosis on pretreatment biopsies using trichrome staining. Post-treatment biopsies were analyzed for fibrosis reversal, and results were compared between patients treated with dietary restriction and those that received topical steroids. Clinical characteristics (age, symptoms, duration of symptoms prior to therapy, treatment type, and duration of therapy) were recorded. Histological markers (eosinophil numbers and eosinophilic degranulation in both epithelium and lamina propria, basal zone hyperplasia, and the presence of eosinophilic microabscesses in the epithelium) were examined by reviewing hematoxylin and eosin-stained biopsies and by immunohistochemical staining. These were examined as potential predictors for fibrosis reversal.

**Results:** Fibrosis resolved following both dietary restriction and topical steroids (3/17 and 5/9 patients respectively,  $P = 0.078$ ). Post-treatment symptom resolution and decreased intraepithelial eosinophil numbers were found to be the only significant predictors of fibrosis resolution.

**Conclusions:** Dietary restriction alone, similar to topical steroids, can reverse fibrosis in children with EoE.

Eosinophilic esophagitis (EoE) is a clinicopathologic disease characterized by eosinophilic infiltration limited to the esophagus in the setting of upper gastrointestinal symptoms despite adequate acid suppression therapy (1). Presenting symptoms include abdominal pain, heartburn/gastroesophageal reflux, and failure to thrive, which are typically seen in children, as well as dysphagia and esophageal food impactions, more commonly seen in adolescents and adults (2–4). The exact pathophysiology of dysphagia and food impactions in patients with EoE is unknown. Currently, there is evidence to suggest that these complications are because of esophageal dysmotility, subepithelial fibrosis, or a combination of these two mechanisms (5–11).

Several reports have now, in fact, demonstrated evidence for increased collagen deposition in the lamina propria (LP) of the esophagus in patients with eosinophilic esophagitis, including in the pediatric population (6, 7, 11). In addition, there is evidence that fibrosis is correlated with clinical and endoscopic findings of EoE. Endoscopic findings of esophageal rings and strictures were seen only in EoE patients with evidence of fibrosis, and food impaction was seen in 24.2% of EoE patients with fibrosis vs only 5.6% of patients without fibrosis (7, 11). Fortunately, this esophageal remodeling appears to be reversible with topical corticosteroid therapy, both in children and adults with EoE (12, 13).

Currently, very little data exist as to whether other forms of therapy for EoE can also reverse subepithelial esophageal fibrosis. Review of the published literature reveals only one report addressing the effect of dietary therapy on fibrosis (14). In this report, fibrosis resolution was seen in one of out patients treated with some form of dietary restriction. Limitations to these data include the very small number of patients treated with dietary restriction alone (two patients) and the fact that these patients concomitantly received antacids without ruling out first gastroesophageal reflux mimicking or compounding EoE, hence confounding the diagnosis and the actual contributor to fibrosis reversal.

The aim of our study was threefold: (i) to determine whether resolution of fibrosis can be achieved with dietary therapy, (ii) to check how dietary therapy compares to corticosteroid therapy in reversing fibrosis, and (iii) to examine for any risk factors associated with lack of fibrosis resolution.

## Materials and methods

This historical cohort study was approved by the Institutional Review Board at the Mount Sinai School of Medicine.

### Patient selection

Medical records of all patients screened and followed for suspected EoE by a single pediatric gastroenterologist (M.C.) at the Mount Sinai Center for Eosinophilic Disorders from January 2005 to July 2010 were reviewed. Patients were included in this study if they met the following criteria:

- All patients had to have pre- and post-therapy upper endoscopies performed by a single pediatric gastroenterologist at the Center, and biopsy specimens obtained in a standardized method for all patients: three distal esophageal biopsies, 3 proximal esophageal biopsies, and additional biopsies of any areas with endoscopic abnormal findings. All biopsies were obtained using Radial Jaw 4, 2.8-mm forceps (Boston Scientific, Natick, MA, USA).
- Gastric and duodenal biopsies obtained from all patients.
- No evidence of variant gastrointestinal anatomy (e.g. gastrectomy).
- First endoscopy (pretherapy) performed by M.C. being the diagnostic one, demonstrating histological evidence of EoE – Patients had to have at least 15 intraepithelial eosinophils per high power field at 400× (HPF) on light microscopic examination despite treatment with high-dose antacid therapy per consensus recommendations (1).
- The presence of adequate LP in the pre- and post-therapy biopsy specimens for assessment of fibrosis (amount of LP that is approximately of the same depth as the mucosa in at least one biopsy).
- LP fibrosis pretherapy.
- No evidence of eosinophilic gastrointestinal disease beyond the esophagus.
- All patients had to have received one form of therapy: either topical corticosteroid therapy in the form of swallowed fluticasone or budesonide, or dietary restriction therapy.

## Histology

All histological evaluations were carried out by one pathologist who was blinded to treatment and clinical history (R.A.M.).

Evaluation of esophageal histologic features – Hematoxylin and eosin (H&E)-stained biopsy slides were retrieved for blind re-evaluation of histology. In the epithelium, the peak number of eosinophils was determined by surveying all biopsy pieces at 10× magnification for the densest eosinophilic infiltration and then counting the peak number of eosinophils per HPF on light microscopy. In the LP, peak eosinophils were counted on H&E-stained sections per HPF.

The presence of intra-epithelial eosinophilic microabscesses was graded as present or absent. Epithelial basal zone hyperplasia (BZH) was scored as the percent of the total epithelial thickness that was occupied by the basal zone in properly oriented specimens using the following, previously published, grading system: 0–20% = 0, 21–33% = 1, 34–66% = 2, and 67–100% = 3 (7).

Evaluation of esophageal eosinophilic degranulation – Additional 3-μm-thick esophageal tissue sections were cut from the paraffin block for immunohistochemical staining for major basic protein (MBP). Briefly, sections were deparaffinized and rehydrated. Antigen retrieval was achieved by enzymatic digestion with trypsin (Sigma-Aldrich, St Louis, MO, USA). Endogenous peroxidase activity was blocked by treatment with hydrogen peroxide. Sections were then incubated with the primary antibody, mouse anti-human MBP, clone BMK-13 (Cell Sciences, Canton, MA, USA), and with mouse IgG1 as its isotype control, followed by a secondary antibody conjugated with a peroxidase-labeled polymer. Diaminobenzidine (DAB) substrate-chromogen solution was used for staining (DAKO, Carpinteria, CA, USA). Hematoxylin was used as a counterstain. Eosinophil degranulation was graded as absent/mild (0 to few extracellular granules) or extensive (moderate to marked degranulation) in the maximally involved areas. Edges of the biopsy specimen and areas with a crushed appearance were excluded from evaluation.

Evaluation of esophageal fibrosis – Additional esophageal tissue sections were also cut from the paraffin block for trichrome staining for collagen determination and evaluation of fibrosis. Fibrosis was defined as densely compacted LP collagen fibers found at any biopsy site. The following pattern was recorded, as previously established (7):

- Normal LP: delicate, lacy collagen with an areolar pattern or coarser collagen, yet individual fibers remain identifiable
- Fibrotic LP: Dense, compact collagen deposition.

Fibrosis resolution was defined as a restoration to normal subepithelial LP architecture (from fibrotic to normal in all the biopsies containing LP) (Fig. 1).

## Statistical analysis

Descriptive statistics are presented as median (inter-quartile range) for non-normally distributed continuous variables and as mean ± SD for those normally distributed. The Wilcoxon rank-sum test or the Student's *t*-test was used to compare continuous variables between treatment groups. The

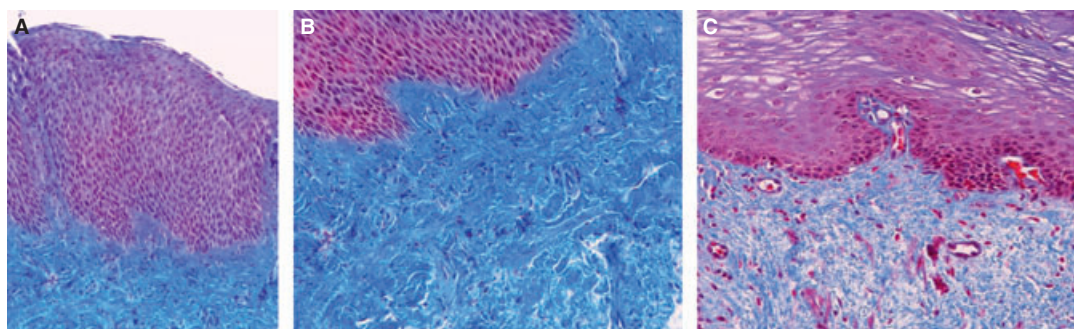


**Figure 1** Trichrome stain of esophageal biopsy sections for fibrosis. (A and B) Esophageal fibrosis: Compact collagen deposition in the lamina propria (original magnification  $\times 100$  and  $\times 200$  respectively).

Wilcoxon matched-pairs rank-sum test was used for between pre- and post-treatment comparisons of the same continuous variables. Associations between categorical data were assessed using the Fisher's exact test. Longitudinal analysis was performed with generalized estimating equations (GEE) to examine the association of fibrosis resolution with each of the independent variables of interest using the binomial family of distribution and an exchangeable correlation structure. The models were adjusted for potential effect modifiers or confounders such as symptom duration, age, and treatment type. The distribution of the variables of interest was assessed with the Shapiro-Wilk test. All reported *P*-values were based on 2-sided tests and compared with a significance level of 5%. Stata 9.1 for Windows (Stata Corp LP, College Station, TX, USA) and GraphPad Prism Version 4.0 (GraphPad Software, Inc., La Jolla, CA, USA) were used for all statistical calculations and plots.

## Results

### Baseline characteristics

Ninety-nine patients with pre- and post-therapy biopsies were identified, 26 of them met all inclusion criteria and were included in the analysis (Fig. 2).

Because patient selection was restricted to a single gastroenterologist (M.C.), all patients received therapy according to a set-established protocol, based on a discussion between the gastroenterologist and patient/caregiver as to whether to receive dietary or steroid therapy, with patient/caregiver lifestyle preference being taken into account. Nine patients were treated with swallowed, topical steroids (eight with fluticasone and one with budesonide), and 17 were treated with dietary restrictions (12 with an empiric elimination diet and five with an amino acid-based formula with one food).

In the fluticasone group, therapy was dosed as follows in all patients (per set protocol, based on a previously published pediatric study) (15): two puffs of fluticasone swallowed twice daily, strength used based on age, 2–4 years old: 44  $\mu\text{g}/\text{puff}$ , 5–11 years old: 110  $\mu\text{g}/\text{puff}$ , and 12 years or older: 220  $\mu\text{g}/\text{puff}$ . One patient received swallowed viscous budesonide therapy at a dose of 0.5 mg twice daily (16). Adherence to medical

therapy was monitored in all patients through regular follow-up visits and prescription monitoring.

Dietary therapy consisted of either an amino acid-based formula plus one food (corn), to which the child had no evidence of IgE-mediated hypersensitivity (as determined by food-specific IgE level and/or prick skin test) or of an empiric elimination diet with the removal of common food allergens (milk, egg, soy, wheat, peanut, tree nuts, seafood, and red meats). If patients had already been avoiding some of these foods because of immediate IgE-mediated allergic reactions, the remaining foods were removed to complete the above-mentioned restriction. Dietary therapy was closely supervised by a dietician with expertise in food allergy in all patients, and adherence was monitored by frequent follow-ups via office visits, phone calls, and e-mails, all of which included food diary analyses. Choice of amino acid-based formula or elimination diet was made based on patient/caregiver preference after discussion with the gastroenterologist and dietician.

The mean  $\pm$  SD age at the time of initial biopsy was  $10.8 \pm 7.2$  years. Twenty-two (84.6%) were male. Abdominal pain was the most frequent presenting symptom, seen in 68% of the patients, followed by nausea/vomiting (44%), dysphagia (36%), food impactions (32%), and failure to thrive (32%). The median peak (IQR) esophageal intraepithelial eosinophil count was 60/HPF (40–90) and that in the LP was 7/HPF (3–16). Median (IQR) treatment duration was 4 (3–5) months. Other clinical and histological features are summarized in Table 1.

The two treatment groups (dietary vs steroid therapy) did not differ with respect to gender, symptom duration, treatment duration, baseline intraepithelial eosinophil count, the presence of intraepithelial or LP eosinophil degranulation, or the presence of eosinophilic microabscesses (Table 1). The steroid-treated group was older (mean age = 14.6 vs 8.7 years,  $P = 0.046$ ), more likely to have symptoms of dysphagia (66.7% vs 18.8%,  $P = 0.031$ ) and food impaction (77.8% vs 6.3%,  $P = 0.001$ ), and had a lower number of LP eosinophils/HPF [median (IQR): 3 (2–6) vs 9 (6–22)] at baseline (Table 1).

The two treatment groups (dietary vs steroid therapy) did not differ with respect to gender, symptom duration, treatment duration, baseline intraepithelial eosinophil count, the presence of intraepithelial or LP eosinophil degranulation, or the presence of eosinophilic microabscesses (Table 1). The steroid-treated group was older (mean age = 14.6 vs 8.7 years,  $P = 0.046$ ), more likely to have symptoms of dysphagia (66.7% vs 18.8%,  $P = 0.031$ ) and food impaction (77.8% vs 6.3%,  $P = 0.001$ ), and had a lower number of LP eosinophils/HPF [median (IQR): 3 (2–6) vs 9 (6–22)] at baseline (Table 1).

### Histological response to therapy

Peak esophageal intraepithelial eosinophil count decreased from a median of 60/HPF to a median of 11/HPF in the entire

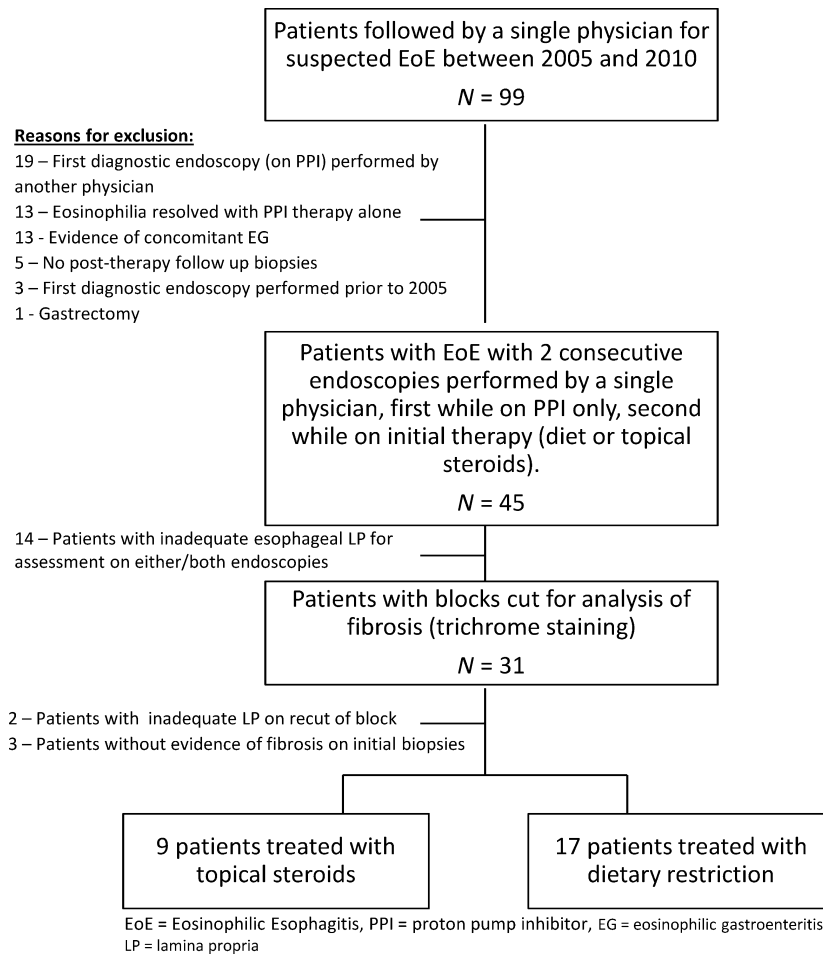


**Figure 2** Flow chart of the patients detailing reasons for exclusion from the study.

group,  $P = 0.001$ . Both steroid therapy and dietary therapy significantly reduced the peak intraepithelial eosinophil count [steroids (median of 50/HPF to 0/HPF,  $P = 0.008$ ); diet (median of 60/HPF to 20/HPF,  $P = 0.019$ )]. However, the group that received steroid therapy had a significantly lower post-treatment intraepithelial eosinophil count as compared to that on dietary therapy,  $P = 0.002$  (Fig. 3).

In the LP, the median peak eosinophil count decreased from 7/HPF to 3/HPF in the entire group,  $P = 0.017$ . In the group receiving dietary therapy, there was a statistically significant decrease in the median peak LP eosinophil count from 9/HPF to 3/HPF ( $P = 0.017$ ). Steroid therapy also tended to decrease the median peak LP eosinophil count (3/HPF to 1/HPF,  $P = 0.482$ ); however, this change was not statistically significant.

#### Fibrosis resolution

Resolution of fibrosis was seen with both treatment modalities. 5/9 (55.6%) patients treated with swallowed steroids had fibrosis resolution vs 3/17 (17.6%) patients who received dietary therapy. However, the difference was not statistically

significant when examining the data in a cross-sectional manner using Fisher's exact test ( $P = 0.078$ ) (Fig. 4).

#### Predictors of fibrosis resolution

We compared all patients who had fibrosis resolution with all those with fibrosis persistence. The two groups did not differ with respect to age, gender, treatment duration, duration of symptoms, history of food impaction, peak intraepithelial eosinophils (pre- or post-treatment), peak LP eosinophils (pre- or post-treatment), the presence of eosinophilic degranulation, the presence of eosinophilic microabscesses, and degree of basal zone hyperplasia (Table 2).

Symptom resolution, defined as resolution of all symptoms, was seen more frequently in those patients that had fibrosis resolution (85.7%) than in those patients that had fibrosis persistence (58.8%). However, this trend was not statistically significant,  $P = 0.352$ .

#### Longitudinal analysis of data

Cross-sectional analysis using common univariate methods like  $t$ -tests and Wilcoxon tests is widely performed in data

**Table 1** Baseline demographics characteristic, symptoms, and histologic findings in all patients

|                                                                                   | All patients ( <i>n</i> = 26)                                                                                                              | Steroid therapy ( <i>n</i> = 9)                                                                                                         | Dietary therapy ( <i>n</i> = 17)                                                                                                         |                                                                                                                                                                   |
|-----------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Age, Mean $\pm$ SD                                                                | 10.8 $\pm$ 7.2 years                                                                                                                       | 14.6 $\pm$ 8 years                                                                                                                      | 8.7 $\pm$ 6.1 years                                                                                                                      | <i>P</i> = 0.046 <sup>‡</sup>                                                                                                                                     |
| Male (%)                                                                          | 22 (84.6%)                                                                                                                                 | 7 (77.8%)                                                                                                                               | 15 (88.2%)                                                                                                                               | <i>P</i> = 0.591 <sup>§</sup>                                                                                                                                     |
| Atopic status                                                                     |                                                                                                                                            |                                                                                                                                         |                                                                                                                                          |                                                                                                                                                                   |
| Rhinoconjunctivitis                                                               | 18 (69.2%)                                                                                                                                 | 5 (55.6%)                                                                                                                               | 13 (76.5%)                                                                                                                               | <i>P</i> = 0.382                                                                                                                                                  |
| Atopic dermatitis                                                                 | 10 (38.5%)                                                                                                                                 | 3 (33.3%)                                                                                                                               | 7 (41.2%)                                                                                                                                | <i>P</i> = 1.000                                                                                                                                                  |
| Food allergy                                                                      | 22 (84.6%)                                                                                                                                 | 6 (66.7%)                                                                                                                               | 16 (94.1%)                                                                                                                               | <i>P</i> = 0.104                                                                                                                                                  |
| Asthma                                                                            | 14 (53.8%)                                                                                                                                 | 3 (33.3%)                                                                                                                               | 11 (64.7%)                                                                                                                               | <i>P</i> = 0.218                                                                                                                                                  |
| Presenting symptoms                                                               | Abdominal pain 17 (68.0%)*<br>Nausea/Vomiting 11 (44.0%)<br>Dysphagia 9 (36.0%)<br>Food impaction 8 (32.0%)<br>Failure to thrive 8 (32.0%) | Abdominal pain 6 (66.7%)<br>Nausea/Vomiting 2 (22.2%)<br>Dysphagia 6 (66.7%)<br>Food impaction 7 (77.8%)<br>Failure to thrive 1 (11.1%) | Abdominal pain 11 (68.8%)*<br>Nausea/Vomiting 9 (56.3%)<br>Dysphagia 3 (18.8%)<br>Food impaction 1 (6.3%)<br>Failure to thrive 7 (43.8%) | <i>P</i> = 1.000 <sup>§</sup><br><i>P</i> = 0.208 <sup>§</sup><br><i>P</i> = 0.031 <sup>§</sup><br><i>P</i> = 0.001 <sup>§</sup><br><i>P</i> = 0.182 <sup>§</sup> |
| Treatment duration, median (IQR)                                                  | 4 months (3–5)                                                                                                                             | 4 months (3–6)                                                                                                                          | 5 months (3–5)                                                                                                                           | <i>P</i> = 0.956 <sup>¶</sup>                                                                                                                                     |
| Duration of symptoms, median (IQR)                                                | 2.0 years (1.3–3)                                                                                                                          | 2.5 years (2–3)                                                                                                                         | 1.8 years (0.5–5)                                                                                                                        | <i>P</i> = 0.550 <sup>¶</sup>                                                                                                                                     |
| Peak esophageal intraepithelial eosinophil count, median (IQR)                    | 60/HPF (40–98)                                                                                                                             | 50/HPF (36–128)                                                                                                                         | 60/HPF (50–88)                                                                                                                           | <i>P</i> = 0.518 <sup>¶</sup>                                                                                                                                     |
| Peak esophageal lamina propria eosinophil count, median (IQR)                     | 7/HPF (3–18)                                                                                                                               | 3/HPF (2–6)                                                                                                                             | 9/HPF (6–22)                                                                                                                             | <i>P</i> = 0.012 <sup>¶</sup>                                                                                                                                     |
| Esophageal basal zone hyperplasia score, mean (IQR)                               | 2.0 (0–3)                                                                                                                                  | 2 (1–2)                                                                                                                                 | 2 (1.5–3)                                                                                                                                | <i>P</i> = 0.212                                                                                                                                                  |
| Presence of eosinophilic degranulation in the esophageal epithelium, <i>n</i> (%) | <i>N</i> = 22 (84.6%)                                                                                                                      | 7 (77.8%)                                                                                                                               | 15 (88.2%)                                                                                                                               | <i>P</i> = 0.591                                                                                                                                                  |
| Presence of eosinophilic degranulation in the esophageal LP, <i>n</i> (%)         | <i>N</i> = 19 (76.0%) <sup>†</sup>                                                                                                         | 6 (75.0%) <sup>†</sup>                                                                                                                  | 13 (76.5%)                                                                                                                               | <i>P</i> = 1.000                                                                                                                                                  |
| Microabscesses in the esophageal epithelium, <i>n</i> (%)                         | <i>N</i> = 16 (61.5%)                                                                                                                      | 4 (44.4%)                                                                                                                               | 12 (70.6%)                                                                                                                               | <i>P</i> = 0.234                                                                                                                                                  |

*P*-values derived after comparing treatment groups.

SD, standard deviation; IQR, inter-quartile range.

\*One patient in the diet group had incomplete records; therefore, the symptom history could not be obtained.

†One patient in the steroid therapy group did not have enough tissue with LP left at initial biopsy after cutting to evaluate for eosinophilic degranulation.

‡Student's *t*-test.

§Fisher's exact test.

¶Wilcoxon rank-sum test.

analysis. However, it does not account for the effect that time has on our outcomes of interest, cannot reveal association with time-dependent variables, and does not take correlations and interactions between variables into consideration. Therefore, while we performed the above cross-sectional analysis for the purpose of comparison with previous studies, we also longitudinally assessed the data (Table 3). After accounting for treatment duration and adjusting for potential confounders or effect modifiers, fibrosis was more likely to disappear as the number of intraepithelial eosinophils decrease (OR: 0.932, *P* = 0.015), whereas the changes in numbers of LP eosinophils did not

seem to play a significant role (OR: 0.832, *P* = 0.143). The effect seen was independent of symptom duration (in the symptom duration-adjusted model, OR: 1.089  $\pm$  0.0416, *P* = 0.026) and type of therapy (in the treatment-adjusted model, OR: 0.075  $\pm$  0.034, *P* = 0.028). Age was neither significantly associated with fibrosis resolution nor played a confounding role in this model.

Similarly, fibrosis was more likely to resolve if the initially observed epithelial eosinophil degranulation disappeared (OR: 0.152  $\pm$  0.132, *P* = 0.029), even after adjusting for the duration of symptoms prior to any type of intervention (OR: 0.088  $\pm$  0.098, *P* = 0.029). However,

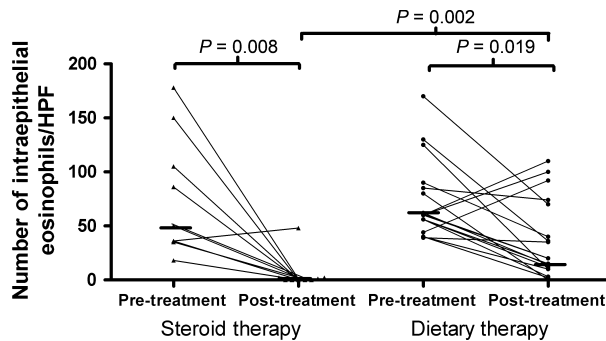


**Figure 3** Histological response based on treatment type.

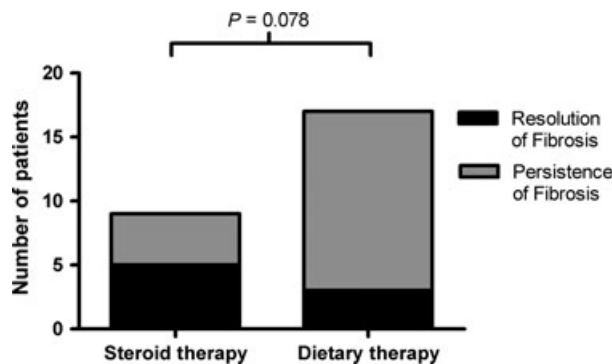


**Figure 4** Resolution of esophageal fibrosis based on treatment type.

this significance disappeared after adjusting for the type of treatment (OR:  $0.2 \pm 0.179$ ,  $P = 0.072$ ) suggesting that when there is a treatment intervention, eosinophil degranulation may not be a significant surrogate marker of fibrosis resolution.

Moreover, while fibrosis reversal and its relation to symptom resolution was not significant in a cross-sectional analysis, we found that fibrosis and symptoms were highly associated with a longitudinal analysis that takes time into account. Fibrosis was expected to resolve after a period of treatment intervention, if all symptoms disappear (OR:  $0.056 \pm 0.063$ ,  $P = 0.011$ ). This effect remained significant even after treatment type (treatment-adjusted model, OR:  $0.036 \pm 0.045$ ,  $P = 0.008$ ) or duration of symptoms (symptom duration-adjusted model, OR:  $0.051 \pm 0.058$ ,  $P = 0.010$ ) were taken into account.

When the effect of treatment type on fibrosis resolution was examined, comparison between the effect of steroids vs diet did not reach statistical significance ( $P = 0.055$ ). However, in the symptom duration (prior to therapy) adjusted model, treatment results became significant (OR:  $0.167 \pm 0.138$ ,  $P = 0.031$ ), favoring steroids.

None of the other covariates (gender, age, duration of symptoms, and basal zone hyperplasia score) was found to be significantly associated with fibrosis resolution (Table 3).

## Discussion

In this pediatric historical cohort study, we have demonstrated that esophageal subepithelial fibrosis can be reversed not only with topical steroid therapy, but also with dietary restriction alone. Fibrosis reversal with the topical steroid budesonide has been demonstrated in children with EoE and fibrosis in a prior report (12). To the best of our knowledge, this is the first study demonstrating fibrosis reversal in response to dietary therapy alone in a series of children. This reversal is unlikely to have occurred spontaneously, because fibrosis was shown to be persistent without therapy for EoE (12, 17).

Because of the absence of a yet validated scoring system to assess fibrosis, we decided to qualify changes in fibrosis as present (pathologic) or absent (normal), rather than assign a fibrosis score. We based this on prior work on fibrosis in patients with EoE and controls, and because the presence of fibrosis has been correlated with changes in endoscopic findings in EoE and with symptoms of dysphagia (7, 11). While a fibrosis score may allow for detection of smaller changes in subepithelial histology, this method has not been validated, and small changes in scores have not been shown to be associated with clinical symptoms or endoscopic findings.

We found that fibrosis reversal was highly associated with resolution of all symptoms when longitudinally analyzing the data. This finding stresses the relevance of fibrosis as present/absent to the clinical picture in children with EoE. It also stresses the importance of considering fibrosis reversal as a treatment goal, not only to prevent long-term irreversible remodeling such as what has been reported in adults, but also to optimize patient's clinical symptoms during their therapy course. It should be noted that there is currently no published, validated symptom-scoring tool available for EoE, and therefore, any report on symptom improvement becomes a limitation in EoE studies. In attempt to minimize this limitation, we used symptom resolution (complete improvement of all symptoms) in an attempt to clearly demarcate symptom improvement.

All but one of our patients in the steroid group received fluticasone. We found fibrosis reversal at a similar rate to that reported in response to budesonide in children (12). In our cohort, 56% patients resolved their fibrosis, compared to 4/8 (50%) children that dropped their fibrosis score to zero in the prior pediatric study (12). These findings are in contrast to what has been observed so far in adults; while the degree of fibrosis was shown to decrease in one study followed prolonged therapy with budesonide (18), fibrosis reversal was not seen (13, 18). These findings suggest that remodeling is more likely to become irreversible in older patients. Within our pediatric population, age did not seem to affect fibrosis reversal. However, it seems that our data combined with previous publications highly suggest that remodeling is likely to be reversed in children rather than in adults.

Next, we compared the effect of dietary restriction vs steroids on fibrosis reversal. While dietary therapy can resolve fibrosis, it appeared that topical corticosteroid therapy may

**Table 2** Characteristics of patients who had esophageal fibrosis resolution vs persistence (cross-sectional analysis)

|                                                                         | Fibrosis resolution<br>( <i>n</i> = 8)             | Fibrosis persistence<br>( <i>n</i> = 18)            | <i>P</i> -value  |
|-------------------------------------------------------------------------|----------------------------------------------------|-----------------------------------------------------|------------------|
| Age, Mean ± SD                                                          | 14.0 years ± 8.4                                   | 9.3 years ± 6.4                                     | <i>P</i> = 0.068 |
| Male (%)                                                                | 6 (75.0%)                                          | 16 (88.9%)                                          | <i>P</i> = 0.563 |
| Treatment duration, median (IQR)                                        | 4 months (3.0–5.5)                                 | 4.5 months (3–5.5)                                  | <i>P</i> = 0.759 |
| Duration of symptoms, median (IQR)                                      | 2.0 years (1.3–3.0)                                | 2.0 years (0.8–5.0)                                 | <i>P</i> = 0.545 |
| History of food impaction prior to treatment, <i>n</i> (%)              | 4 (57.1%)*                                         | 4 (22.2%)                                           | <i>P</i> = 0.156 |
| Resolution of symptoms, <i>n</i> (%)                                    | 6 (85.7%)†                                         | 10 (58.8%)‡                                         | <i>P</i> = 0.352 |
| Peak esophageal intraepithelial eosinophil count, median (IQR)          | Pretreatment<br>59/HPF (36–108)                    | Pretreatment<br>60/HPF (44–90)                      | <i>P</i> = 0.739 |
|                                                                         | Post-treatment<br>2/HPF (0–13)<br><i>P</i> = 0.012 | Post-treatment<br>18/HPF (2–70)<br><i>P</i> = 0.017 | <i>P</i> = 0.066 |
| Peak esophageal lamina propria eosinophil count, median (IQR)           | Pretreatment<br>3/HPF (2–8)                        | Pretreatment<br>8/HPF (3–18)                        | <i>P</i> = 0.059 |
|                                                                         | Post-treatment<br>0/HPF (0–7)<br><i>P</i> = 0.149  | Post-treatment<br>3/HPF (1–6)<br><i>P</i> = 0.044   | <i>P</i> = 0.285 |
| Esophageal basal zone hyperplasia score, Mean ± SD                      | Pretreatment<br>1.8 ± 1.3                          | Pretreatment<br>2.0 ± 0.8                           | <i>P</i> = 0.552 |
|                                                                         | Post-treatment<br>0.9 ± 1<br><i>P</i> = 0.033      | Post-treatment<br>1.1 ± 1<br><i>P</i> = 0.009       | <i>P</i> = 0.677 |
| Presence of eosinophilic degranulation in the esophageal epithelium     | Pretreatment<br><i>N</i> = 6 (75.0%)               | Pretreatment<br><i>N</i> = 16 (88.9%)               | <i>P</i> = 0.563 |
|                                                                         | Post-treatment<br><i>N</i> = 2 (25.0%)             | Post-treatment<br><i>N</i> = 9 (50%)                | <i>P</i> = 0.395 |
| Presence of eosinophilic degranulation in the esophageal lamina propria | Pretreatment‡<br><i>N</i> = 6 (85.7%)              | Pretreatment‡<br><i>N</i> = 13 (54.2%)              | <i>P</i> > 0.999 |
|                                                                         | Post-treatment‡<br><i>N</i> = 2 (28.6%)            | Post-treatment‡<br><i>N</i> = 7 (41.2%)             | <i>P</i> = 0.669 |
| Presence of eosinophilic microabscesses in the esophageal epithelium    | Pretreatment<br><i>N</i> = 3 (37.5%)               | Pretreatment<br><i>N</i> = 13 (72.2%)               | <i>P</i> = 0.189 |
|                                                                         | Post-treatment<br><i>N</i> = 0 (0.0%)              | Post-treatment<br><i>N</i> = 7 (38.9%)              | <i>P</i> = 0.062 |

\*One patient in the fibrosis resolution group did not have information regarding food impaction in the medical record.

†For one patient in each group, symptom resolution could not be obtained from records.

‡One patient in each group did not have enough tissue left in both pre- and post-therapy biopsies to cut additional sections for evaluation of eosinophilic degranulation.

be more effective than dietary therapy in this regard when children are followed up over a short period of time, although this trend did not reach statistical significance in either the cross-sectional (Fig. 3, *P* = 0.078) or the longitudinal (Table 3, *P* = 0.055) approaches. When symptom duration (prior to treatment initiation) was taken into account however, the treatment effect on fibrosis resolution became significant in favor of steroids (*P* = 0.031).

A possible explanation for the trend toward improved fibrosis reversal with corticosteroids may simply be the superiority of topical corticosteroids in resolving tissue eosinophilia in this cohort, an interesting finding in this noncontrolled comparison, suggesting that, at least in this cohort, topical corticosteroids are superior to dietary therapy in resolving tissue eosinophilia. In fact, when we examined for various predictors for fibrosis resolution using a longitudinal approach, we were able to uncover that

improvement in epithelial eosinophilia was significantly associated with fibrosis reversal. This observation is in line with the previous findings shown by Aceves et al. in a pediatric population treated with budesonide, and more recently by Kagalwalla et al. (12, 19). In fact, Kagalwalla et al. (19) also showed that epithelial mesenchymal transition, a process thought to be involved in remodeling, improved with both dietary and corticosteroid therapy, and that the improvement was directly associated with improvement in epithelial eosinophilia. We also found that resolution of eosinophilic degranulation in the epithelium was associated with fibrosis resolution when adjusted for symptom duration. This supports our earlier findings of an association between fibrosis and intraepithelial eosinophil degranulation (7) and supports the theory that eosinophils are not simply innocent bystanders seen on biopsies from patients with EoE, but rather play an integral role in disease pathogenesis (20). It is clear that

**Table 3** Generalized estimating equation models for fibrosis changes during the course of the treatments applied

| Independent variables                                                      | Fibrosis resolution<br>(dependent variable) |         |
|----------------------------------------------------------------------------|---------------------------------------------|---------|
|                                                                            | Odds ratios $\pm$ SE                        | P-value |
| Gender<br>(male vs female)                                                 | 2.111 $\pm$ 1.767                           | 0.372   |
| Age (years)                                                                | 1.079 $\pm$ 0.053                           | 0.122   |
| Symptom duration<br>(months)                                               | 1.017 $\pm$ 0.016                           | 0.304   |
| Treatment<br>(using steroids vs diet)                                      | 0.252 $\pm$ 0.181                           | 0.055   |
| Symptom resolution<br>(from having symptoms to not having)                 | 0.056 $\pm$ 0.063                           | 0.011   |
| Histopathological parameters                                               |                                             |         |
| Intraepithelial eosinophils<br>(for every one eosinophil decrease)         | 0.932 $\pm$ 0.027                           | 0.015   |
| Lamina propria eosinophils<br>(for every one eosinophil decrease)          | 0.832 $\pm$ 0.104                           | 0.143   |
| Epithelial eosinophil degranulation<br>(from existing to disappearing)     | 0.152 $\pm$ 0.132                           | 0.029   |
| Lamina propria eosinophil degranulation<br>(from existing to disappearing) | 0.263 $\pm$ 0.233                           | 0.132   |
| Basal zone hyperplasia score<br>(for every one unit decrease)              | 0.541 $\pm$ 0.215                           | 0.123   |

Data are presented as odds ratios  $\pm$  standard error (SE). The data are estimates of the coefficients for the models with fibrosis as dependent variable at the two time points of fibrosis assessment based on a generalized estimating equations (GEE) model to examine the fibrosis association with each of the independent variables listed using the binomial family of distribution and an exchangeable correlation structure. Convergence was not achieved in the models assessing the effect of eosinophilic microabscesses in the epithelium and history of food impaction on fibrosis resolution.

eosinophilic activation is not the only requirement for subepithelial fibrosis though. Lucendo et al. (18) recently demonstrated that EoE patients with fibrosis had upregulation of certain cytokines, IL-5, FGF-9, and CCL18, suggesting that a complex interplay of multiple immune cells is likely involved in fibrosis.

As with other historical cohort studies, our study has its limitations. Comparable to all the previous studies, our sample size is limited by the rarity of the disease and the stringent inclusion criteria, given that procurement of LP is not expected in endoscopically obtained esophageal biopsies. Nonetheless, we were able to include a relatively larger cohort of patients than in the previous studies examining for esophageal fibrosis pre- and post-therapy. Therapy options were not randomized as would be carried out in a prospective study. However, therapy options in all patients were left to the discretion of the patient/caregiver. In addition, the two groups (dietary therapy vs steroid therapy) were similar with respect to duration of symptoms and duration of therapy, as well as baseline esophageal intraepithelial eosinophilia,

eliminating any potential influence from these parameters. The group that received topical steroids was generally older and had a higher proportion of children with dysphagia. However, we have previously found esophageal fibrosis in very young children and demonstrated that fibrosis formation was not related to age in the pediatric population (7). Furthermore, fibrosis is not limited to patients with dysphagia and can rather be present in children presenting with any upper gastrointestinal symptoms (7, 12). In addition, we did not find age to influence fibrosis reversal in our longitudinal analysis model. Therefore, the above differences are not likely to alter the relevance of our results. Perhaps more importantly, the fact that those patients treated with dietary therapy had higher levels of LP eosinophils prior to therapy, yet still demonstrated fibrosis reversal, highlights the finding that dietary therapy can indeed resolve fibrosis.

In addition, as with all studies examining pre- and post-therapy biopsy specimens and comparing differences, there is a risk that sampling techniques, depth of biopsies, location of biopsies, and number of biopsies could differ between endoscopies even for the same patient and therefore alter the results. In our cohort, because all procedures and therapies were performed as part of a set protocol, these limitations were overcome. All biopsies were obtained by a single gastroenterologist who took the same number of biopsies in a systematic manner each time, and then, slides were all read by a single pathologist blinded to the data including therapy options and timing with respect to therapy. One hypothetical possibility is that the severity of fibrosis may affect the ease of obtaining biopsies. The fact that suction was applied perpendicularly to the esophageal mucosa when procuring the biopsy specimens rendered this less likely, because not only more than 50% of our esophageal biopsies had LP (Fig. 2), but we were also able to identify several patients with severe fibrosis in our cohort.

In conclusion, dietary therapy alone, similar to topical steroid therapy, can resolve subepithelial esophageal fibrosis in EoE, although this effect was not the case for all patients. It appears that topical steroids may be more effective in restoring normal LP tissue architecture upon short-term therapy. Post-treatment symptom resolution and decreased intraepithelial eosinophil numbers are significant predictors of fibrosis resolution. Large multi-center prospective studies are needed to verify these findings. In addition, longitudinal follow-up studies are needed to assess the duration of dietary restriction that is necessary to achieve similar rates of fibrosis reversal, if any, as seen with steroids.

## Funding

This project was funded in parts by the Buckeye Foundation and the Food Allergy Initiative (to M.C.).

## Conflict of interest

The authors have no conflicts of interest to disclose.

## References

1. Liacouras CA, Furuta GT, Hirano I, Atkins D, Attwood SE, Bonis PA et al. Eosinophilic esophagitis: updated consensus recommendations for children and adults. *J Allergy Clin Immunol* 2011;**128**:3–20.
2. Spergel JM, Brown-Whitehorn TF, Beausoleil JL, Franciosi J, Shuker M, Verma R et al. 14 years of eosinophilic esophagitis: clinical features and prognosis. *J Pediatr Gastroenterol Nutr* 2009;**48**:30–36.
3. Aceves SS, Newbury RO, Dohil MA, Bastian JF, Dohil R. A symptom scoring tool for identifying pediatric patients with eosinophilic esophagitis and correlating symptoms with inflammation. *Ann Allergy Asthma Immunol* 2009;**103**:401–406.
4. Dellon ES, Gibbs WB, Fritchie KJ, Rubinas TC, Wilson LA, Woosley JT et al. Clinical, endoscopic, and histologic findings distinguish eosinophilic esophagitis from gastroesophageal reflux disease. *Clin Gastroenterol Hepatol* 2009;**7**:1305–1313.
5. Lucendo AJ, Castillo P, Martin-Chavarri S, Carrion G, Pajares R, Pascual JM et al. Manometric findings in adult eosinophilic esophagitis: a study of 12 cases. *Eur J Gastroenterol Hepatol* 2007;**19**:417–424.
6. Aceves SS, Newbury RO, Dohil R, Bastian JF, Broide DH. Esophageal remodeling in pediatric eosinophilic esophagitis. *J Allergy Clin Immunol* 2007;**119**:206–212.
7. Chehade M, Sampson HA, Morotti RA, Magid MS. Esophageal subepithelial fibrosis in children with eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* 2007;**45**:319–328.
8. Korsapati H, Babaei A, Bhargava V, Dohil R, Quin A, Mittal RK. Dysfunction of the longitudinal muscles of the oesophagus in eosinophilic oesophagitis. *Gut* 2009;**58**:1056–1062.
9. Nurko S, Rosen R, Furuta GT. Esophageal dysmotility in children with eosinophilic esophagitis: a study using prolonged esophageal manometry. *Am J Gastroenterol* 2009;**104**:3050–3057.
10. Aceves SS, Chen D, Newbury RO, Dohil R, Bastian JF, Broide DH. Mast cells infiltrate the esophageal smooth muscle in patients with eosinophilic esophagitis, express TGF- $\beta$ 1, and increase esophageal smooth muscle contraction. *J Allergy Clin Immunol* 2010;**126**:1198–1204.
11. Li-Kim-Moy JP, Tobias V, Day AS, Leach S, Lemberg DA. Esophageal subepithelial fibrosis and hyalinization are features of eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* 2011;**52**:147–153.
12. Aceves SS, Newbury RO, Chen D, Mueller J, Dohil R, Hoffman H et al. Resolution of remodeling in eosinophilic esophagitis correlates with epithelial response to topical corticosteroids. *Allergy* 2010;**65**:109–116.
13. Straumann A, Conus S, Degen L, Felder S, Kummer M, Engel H et al. Budesonide is effective in adolescent and adult patients with active eosinophilic esophagitis. *Gastroenterology* 2010;**139**:1526–1537.
14. Abu-Sultaneh SM, Durst P, Maynard V, Elitsur Y. Fluticasone and food allergen elimination reverse sub-epithelial fibrosis in children with eosinophilic esophagitis. *Dig Dis Sci* 2011;**56**:97–102.
15. Teitelbaum JE, Fox VL, Twarog FJ, Nurko S, Antonioli D, Gleich G et al. Eosinophilic esophagitis in children: immunopathological analysis and response to fluticasone propionate. *Gastroenterology* 2002;**122**:1216–1225.
16. Dohil R, Newbury R, Fox L, Bastian J, Aceves S. Oral viscous budesonide is effective in children with eosinophilic esophagitis in a randomized, placebo-controlled trial. *Gastroenterology* 2010;**139**:418–429.
17. Straumann A, Spichtin HP, Grize L, Bucher KA, Beglinger C, Simon HU. Natural history of primary eosinophilic esophagitis: a follow-up of 30 adult patients for up to 11.5 years. *Gastroenterology* 2003;**125**:1660–1669.
18. Lucendo AJ, Arias A, De Rezende LC, Yague-Compadre JL, Mota-Huertas T, Gonzalez-Castillo S et al. Subepithelial collagen deposition, profibrogenic cytokine gene expression, and changes after prolonged fluticasone propionate treatment in adult eosinophilic esophagitis: a prospective study. *J Allergy Clin Immunol* 2011;**128**:1037–1046.
19. Kagalwalla AF, Akhtar N, Woodruff SA, Rea BA, Masterson JC, Mukkada V et al. Eosinophilic esophagitis: epithelial mesenchymal transition contributes to esophageal remodeling and reverses with treatment. *J Allergy Clin Immunol* 2012;**129**:1387–1396.
20. Straumann A, Aceves SS, Blanchard C, Collins MH, Furuta GT, Hirano I et al. Pediatric and adult eosinophilic esophagitis: similarities and differences. *Allergy* 2012;**67**:477–490.