

Evidence for primary laryngeal inhalant allergy: a randomized, double-blinded crossover study

Douglas F. Roth, MM, MA, CCC-SLP^{1,2}, Katherine Verdolini Abbott, PhD, CCC-SLP^{1,2},
Thomas L. Carroll, MD³ and Berrylin J. Ferguson, MD, FACS, FAAOA⁴

Background: Despite anecdotal reports, no controlled studies to date link allergen exposure with a change in vocal function or dysphonia. The aim of this study was to determine whether allergen exposure in susceptible individuals impairs vocal function.

Methods: The study was a prospective, double-blind, placebo-controlled study in which subjects serve as their own controls. The participants were 5 inhalant allergic adults with suspected dysphonia from allergies, without evidence of reactive lower airways based on methacholine challenge. All subjects were exposed to 2 experimental conditions in which they were challenged with (1) orally inhaled diluent placebo on 1 day, and (2) orally inhaled allergen on another day. Conditions were randomly ordered across subjects and separated by at least 48 hours. Phonatory threshold pressure (PTP) at the 80th percentile pitch was measured prior to diluent and allergen challenge, and 15 and 60 minutes postchallenge to assess potential change in vocal function after challenge testing.

Results: A repeated measures ANOVA revealed a significant main effect for treatment (allergen vs placebo, $p = 0.013$) with greater PTP required post-allergen challenge compared to placebo and an effect size of 0.821.

Conclusion: A primary causal relationship between allergen exposure and impaired vocal function, as assessed by PTP, was observed in adults with documented allergy independent of asthma or nasal exposure. The current design establishes a safe model for laryngeal inhalant allergen challenge.
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Key Words:

laryngeal allergy; allergy; voice; dysphonia; laryngitis; phonatory threshold pressure (PTP)

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Clinicians and professional voice users widely assume a strong connection exists between allergies and voice

¹University of Pittsburgh, School of Health and Rehabilitation Sciences, Department of Communication Science and Disorders, Pittsburgh, PA;

²University of Pittsburgh Medical Center (UPMC), Department of Otolaryngology, UPMC Voice Center, Pittsburgh, PA; ³The Center for Voice and Swallowing, Department of Otolaryngology, Tufts Medical Center, Boston, MA; ⁴University of Pittsburgh Medical Center, Department of Otolaryngology, Division of Sino-Nasal Disorders and Allergy, Pittsburgh, PA

Correspondence to: Berrylin J. Ferguson, MD, FACS, FAAOA, Department of Otolaryngology, Division of Sinonasal Disorders and Allergy, University of Pittsburgh Medical Center, Suite 500, 200 Lothrop Street, Pittsburgh, PA 15213; e-mail: fergusonbj@upmc.edu

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problems. Patients with documented or suspected allergies complain of multiple vocal difficulties such as reduced upper pitch range, lack of vocal clarity, difficulty with register transitions, excessive mucus, vocal fatigue, strain, increased effort to produce voice, and dysphonia. Over the past 40 years an association between allergies and vocal impairment has been reported in numerous anecdotal reports, case studies, uncontrolled prospective trials, and retrospective studies.^{1–20} Currently, vocal impairment from allergies is usually attributed to the secondary effects of allergic rhinitis, postnasal drip, asthma, or medications used in treating upper and lower airway allergies.^{21–30} To date, no placebo-controlled studies have shown that direct laryngeal allergen challenge produces dysphonia. This is not because of lack of attempts, but because of inability to safely conduct a laryngeal allergen challenge study.

Four crucial design challenges unresolved in previous laryngeal allergen models in humans include: (1) avoidance of nose and sinus exposure to allergen to prevent secondary mechanisms potentially contributing to vocal changes; (2) avoidance of lower airway reaction with inhaled allergen

challenge and potential side effects from precipitation of asthma; (3) selection of safe and adequate allergen dose likely to elicit an allergic laryngeal response; and (4) identifying a reliable and quantifiable outcome measure sensitive to anticipated allergic laryngeal changes and associated decline in vocal function. To date, no study has addressed all 4 methodological concerns. We present the difficulties encountered in previous studies surrounding these 4 issues and how they were addressed in the present study.

First, isolating the larynx from sinonasal allergic responses was partially addressed by Dworkin et al.¹² and Reidy et al.,¹⁷ using a nebulized orally inhaled laryngeal allergen provocation method. Moreover, oral inhalation of allergen with the use of a nose clip, as in our study, performed according to the American Thoracic Society recommendations, insured that inspiration of the allergen bypassed the nose.³¹ However, in the previous studies,^{12,17} lower airway responses were not eliminated since asthmatics were not excluded using the most sensitive method of determining lower airway bronchial reactivity, a methacholine challenge. Asthmatic symptoms include cough, which can cause mechanical trauma to the vocal folds and transport lower airway secretions to the larynx, either of which may alter vocal functioning and possibly induce dysphonia.^{1,12,17} According to the American Thoracic Society, methacholine challenge testing (MCT) has strong predictive power for excluding a diagnosis of asthma.³² Therefore, we required a negative methacholine challenge in all subjects prior to entry, minimizing the potential for asthma symptoms to confound the results.

Second, there are significant risks associated with testing the allergic response of the larynx in humans.³³ The potentially fatal complications of nebulized laryngeal antigen challenge were highlighted in a recent study in which the first 2 subjects experienced serious pulmonary reactions necessitating termination of any additional trials.¹² Although the study excluded subjects with reported asthma, no formal testing was done to evaluate airway reactivity. To eliminate inclusion of occult bronchial reactivity we required a negative methacholine challenge as an entry requirement.

Third, the identification of appropriate antigen dosing must be (1) sufficient to provoke a measureable allergic response but (2) lower than a dose which could induce pulmonary complications. In at least 1 reported case¹⁷ the lack of any measureable laryngeal findings after allergen challenge was partly attributed to insufficient allergen dose (200:1 concentration; 5-minute exposure).¹⁷ A later study employed a higher concentration of allergen (40:1 concentration; 5-minute exposure) but had to be aborted after provoking bronchial reactivity in the first 2 subjects.¹² To establish an adequate yet safe dose of antigen for use in the double-blind portion of the study, subjects completed dose escalation trials. During these trials, the subjects' vocal function and respiratory status were monitored to identify the minimum dose of nebulized allergen effecting a change in vocal function without impacting respiratory status.

Fourth, the establishment of a sensitive, objective outcome measure has eluded several prior investigations.^{1,2,15,17} Some prior studies reporting negative results have used perceptual and acoustic measures of questionable reliability and validity,^{34–38} which may have precluded a positive result from the outset. Our study addressed this problem by using an outcome measure shown in several other studies to be sensitive to the expected vocal edema and increased viscosity of laryngeal tissues and secretions—phonatory threshold pressure (PTP).^{39–41}

In sum, although preliminary evidence suggests the larynx may be independently capable of producing an allergic response, recent attempts to experimentally test this hypothesis have been thwarted by several design problems. Our current model, successfully overcame these previous obstacles by (1) reducing the likelihood of sinonasal exposure; (2) eliminating potential bronchial reactivity that might preclude safe completion; (3) establishing a safe and adequate allergen dose using an unblinded titration method, prior to blinded study initiation; and finally (4) by utilizing a validated outcome measure sensitive to how the expected laryngeal changes would impact vocal function.

Subjects and methods

Study design and oversight

A double-blind, placebo-controlled crossover design was used to study the effect of allergen inhalation on vocal function in nonasthmatic allergic adults. The study was performed in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of the University of Pittsburgh. All subjects provided written informed consent before entering the study.

The primary endpoint was change from baseline in PTP at each subject's 80th percentile pitch in the semitone pitch range, described shortly, following nebulized allergen compared to placebo inhalation.

Participants

Inclusion criteria for participation in the study were as follows: (1) nonasthmatic individuals between the ages of 18 to 70 years with a history of "allergic laryngitis" based on a positive response to at least 1 of 4 questions developed by Jackson-Menaldi (Table 1)¹⁶; (2) who, following

TABLE 1. Jackson-Menaldi allergic history questions*

1	Have you ever had, or do you now have yearly hay fever during any predictable pollen allergy seasons?
2	Have you ever had a violent reaction around a cat?
3	Have you ever had a reaction when cleaning out a basement, garage or when vacationing in an old cottage?
4	Do you have a family history of allergy?

*Initial screening questions asked of potential subjects before proceeding to initial screening day.

TABLE 2. Dose establishment day (day 2) results showing the antigen concentration and number of puffs that resulted in a 2 SD increase in PTP from each subject’s baseline mean on the PTP task*

Subject	Gender	Nebulized antigen	Concentration administered	Number of puffs delivered	80th percentile pitch used for PTP
1	F	Ragweed	1000:1	4	B ₄ , 494 Hz
2	F	Cat	100:1	1	C ₅ , 523 Hz
3	F	Cat	10:1	1	D ₅ , 587 Hz
4	F	Cat	10:1	4	E ^b ₅ , 622 Hz
5	F	Cat	100:1	4	B ₄ , 494 Hz
6 ^a	F	Cat	10:1	4	A ^b ₅ , 831 Hz

*The antigen dosing and established 80th percentile pitch were used for the 2 subsequent double-blinded experimental days.

^aSubject 6 did not proceed to experimental days 3 and 4.

F = female; PTP = phonation threshold pressure; SD = standard deviation.

initial screening, had both a positive allergen prick screen and intradermal allergy wheal confirmation, and a negative methacholine challenge performed according to standard protocol³²; (3) were without any structural or neurologic laryngeal abnormality based on consensus videostroboscopic evaluation by a laryngologist and speech-language pathologist; and (4) declared ability and willingness to participate in the timed protocol as scheduled. Of 45 subjects who responded to an advertisement for the study, 13 met initial screening criteria and were consented. Of these, 8 failed to complete the study for the following reasons: 2 did not show a wheal to ≥ 3 endpoint dilution of antigen on intradermal allergen testing (a 7-mm wheal response to a concentration of allergen at the 1:2500 dilution, with both a positive histamine wheal and a negative diluents wheal), 2 had vocal lesions, 2 were unable to commit to time required (4 full days of testing), 1 had a positive methacholine challenge, and 1 (subject 6) previously treated in our clinic and known to have allergies failed to demonstrate a meaningful voice change on the dose establishment day and was not advanced to the double blinded portion of the study. Five subjects were enrolled and completed the study (Table 2). Because of the strong symptomatic overlap between laryngopharyngeal reflux (LPR) and potential laryngeal allergy symptoms, subjects could not specifically be excluded for LPR signs or symptoms. The results below were obtained in specific blocks of time when subjects were not permitted to break for food or drink (except water) in an effort to prevent any postprandial laryngeal changes from LPR.

Primary outcome measures

The primary outcome measure was estimated PTP in cm/H₂O. This measure reflects noninvasively the minimum subglottic pressure required to initiate and sustain phonation. Theoretically and empirically, PTP has been shown to be regulated by the following factors⁴²:

$$PTP = (2k/T) (B) (c) w/2$$

[1]

where *k* is a constant equal to 1.1, *T* is the vertical thickness of the vocal folds, *B* is a damping coefficient related to vocal fold tissue viscosity, *c* is the speed of mucosal wave propagation related to tissue stiffness, and *w*/2 is the prephonatory glottal half-width at the vocal processes of the arytenoids. Relevant for the present study, PTP varies directly with tissue mass and epithelial thickening,⁴¹ as well as with tissue viscosity, which are all changes expected to occur in the allergic response.^{12,15,17} PTP modulation with various interventions is expected to be particularly pronounced at the high pitches (which are produced with elongated, stiff vocal folds) used in this study compared to lower pitches (which are produced with shorter, lax vocal folds) due to the greater impact of increases in viscosity inherent to elongated tissue.^{41,43} PTP data were collected, as described below using the Kay Aerophone II (Kay Elemetrics Corp., Montvale, NJ), DOS version March 24, 2002. Equipment was calibrated daily during the study, per the manufacturer’s instructions.

Intervention

Study day 1 (screening)

On day 1, subjects underwent allergy prick screen and methacholine challenge administered by a KOKO dosimeter (nSpire Health, Inc., Longmont, CO), using pulmonary function testing (PFT) to exclude individuals with lower airway reactivity. Intradermal allergy testing was also completed.

Study day 2 (initial data collection and establishment of minimal inhaled antigen dose affecting vocal change)

On study visit day 2, each subject first completed PFT. For PFTs, the subject took 3 tidal breaths followed by a maximal inspiration and a forced maximal exhalation. Resulting data were used to establish forced expiratory volume (FEV1) in the first second of exhalation. These trials were repeated until 3 exemplars yielded results within 150 mL of each other.

TABLE 3. Progression of antigen dosing used on successive inhalation challenge trials used on the dose escalation day to safely establish the smallest dose that would affect a ≥ 2 SD increase in PTP

Trial	1	2	3	4	5	6
Nebulized antigen concentration	1000:1	1000:1	100:1	100:1	10:1	10:1
Number of 0.6-second puffs administered	1	4	1	4	1	4

PTP = phonation threshold pressure; SD = standard deviation.

Next, we established the subject's semitone pitch range using ascending and descending pitch glides on /a/, on repeated trials, until reliable minimum and maximum pitches were produced both to the examiner and subject's approval. From the corresponding semitone pitch range, the 10th and 80th percentile pitches were calculated. The established pitches would be used for all PTP data collection events, for that subject, in the study.

Next, the subject was trained in PTP procedures. Specifically, as indicated in studies examining reliability and validity of PTP measures, the subject was trained to produce strings of /pi pi pi pi pi/ as quietly as possible, at a rate of 92 beats per minute in synchrony with a metronome at both of the subject's target pitches,⁴⁴ using the Kay Aerophone II. Care was taken to assure the subject achieved and maintained a firm seal between the Aerophone face mask and face throughout all trials. If an attempt exceeded threshold, in the examiner's judgment, then the syllable string was repeated until satisfactory performance was achieved ($<5\%$ of trials). Once the subject demonstrated competence in the procedures (which usually took about 5 minutes to master), he or she proceeded to produce 5 repetitions of the 5-syllable string, for a total of 3 sets, with each set separated by 15-minute rest intervals. These data were used to establish the subject's baseline values, as described in Data Reduction. Two secondary measures were collected at the time of the experiment but pertain to another question unrelated to allergy specifically and will be presented in another venue: (1) PTP at the 10th percentile pitch and (2) direct magnitude estimation (DME) rating of the subjects' self-perceived effort in producing the 3 sets of /pi pi pi pi pi/ syllable strings.

After PTP baseline data were collected, we established individualized subject-specific doses of nebulized antigen required to produce PTP changes. Based on allergen prick screen, the antigen with the largest wheal was utilized for transoral nebulized antigen challenge using a dosimeter in a manner similar to methacholine challenge, including the use of a nose clip. Specifically the dose was escalated using the schedule in Table 3. Antigen challenge was begun using the most dilute inhalation. FEV1 was measured at baseline and 3 minutes after *every* antigen challenge. Dose escalation continued until 1 of 2 a priori criteria were met: (1) average PTP for 5 repetitions of 5-syllable string met or exceeded 1 standard deviation (SD) above the baseline mean for that subject, 15 minutes after antigen exposure or (2) the subject's FEV1 dropped 20% below baseline. Any subject whose FEV1 dropped 20% or more would be

excluded from further participation, which would indicate lower airway activity. This event did not occur for any of the subjects in the study. The concentration of antigen required to produce at least a 1 SD increase in PTP was utilized in the subsequent double-blind portions of the study as the antigen dose delivered. Of the 6 subjects who completed day 2 of the study, 1 failed to exceed 1 SD from PTP baseline and was dropped from further participation. The implications of her data will be discussed.

Study day visit 3 and 4 (double-blind crossover study)

All subjects in whom an allergen caused a significant increase in PTP continued to study day 3, which was at least 48 hours and not more than 3 weeks following study visit 2. A new complete set of baseline PTP and FEV1 data was collected on days 3 and 4. Then the subject was challenged in double-blind fashion with either diluent placebo or the dose of antigen that had caused a 1 SD increase in PTP on study visit 2. FEV1 data collection followed 3 minutes after exposure. PTP data were obtained 15 minutes and 60 minutes postchallenge. Based on random assignment, some subjects received placebo diluent and some subjects received antigen on day 3. Subjects then received the alternate administration on day 4. Study visit 4 occurred at least 48 hours and not more than 3 weeks after visit day 3. Timelines for all aspects of the procedures are shown in Table 4.

Data management

Data reduction

As noted, new PTP baseline measures were extracted on each day of the study. The purpose was to account for day to day intrasubject variability in the measures. PTP data used in statistical analyses were obtained from the third baseline trial set (including 5 repetitions of the 5-syllable string) and from the 15-minute and 60-minute postchallenge time points (similarly including 5 repetitions of the 5-syllable string) from days 3 and 4. For these data sets, the first and last syllables from each 5-syllable string were excluded from analysis. For the remaining 15 syllables in the set, PTP was measured manually from raw data saved in the Aerophone system. The mean and SD across all 15 syllables was used in further data management.

For further management, mean subglottic pressure scores thus obtained from each time point from each day were

TABLE 4. Sequence and timing of procedures used on the antigen and placebo challenge days (randomly ordered days 3 and 4)*

Time	Procedure
0:00	Baseline PFT (FEV1)
10:00	Establish pitch range
15:00	Baseline PTP set 1
	Rest
30:00	Baseline PTP set 2
	Rest
45:00	Baseline PTP set 3
50:00	Inhalant challenge (placebo or antigen)
	3-minute rest
53:00	PFT for airway safety monitoring
	Rest
1:05:00	15-minute postchallenge PTP set
	Rest
1:50:00	1-hour postchallenge PTP set

*Subjects started the procedures at the same time of day for days 3 and 4.
FEV1 = forced expiratory volume; PFT = pulmonary function testing; PTP = phonation threshold pressure.

transformed into Z-scores defined as:

$$Z = (x - B)/SD \tag{2}$$

where *x* is the average subglottic pressure from a given postchallenge time point on a given day, *B* is the average pressure from the third baseline set on that day, and *SD* is the standard deviation from that day’s baseline data.

Data analyses

Z-scores thus obtained were entered into a 2 × 2 within subjects repeated measures analysis of variance (ANOVA). Independent variables were time point (15-minutes and 60-minutes postchallenge) and exposure (antigen vs placebo). The dependent variable was PTP Z-score. Preliminary analyses confirmed assumptions required for repeated measures ANOVA were met. Effect sizes were also obtained for the data and power analyses conducted. In addition to statistical analyses, raw data were examined without Z-score transformation.

Results

General observations

During the dose establishment day, no subject experienced any shortness of breath secondary to inhalation of the nebulized antigen. Similarly, at no time did any subject’s FEV1 approach a 20% drop below baseline FEV1. All of the 5 subjects whose subglottic pressures increased at least 1 SD from their baseline PTP means went on to complete the 2 double-blinded experimental days. A sixth subject failed to

demonstrate an increase of 1 SD from baseline PTP even after reaching the maximum established dose of 10:1 concentration of cat antigen with a 4-puff exposure. This subject had a documented history of allergy testing from our clinic with a wheal to >3 endpoint dilution for cat antigen on intradermal allergen testing. She had been treated with immunotherapy in the past but had not received any treatments within the year prior to being enrolled in the study. After the subject failed to demonstrate a laryngeal response to the nebulized cat antigen, a standard intradermal test for cat was repeated but failed to show a significant wheal at endpoint dilution 3. The implications of these findings are explored in the discussion. The antigen with which each subject was challenged as well as the antigen concentration and number of puffs required to cause a 1 SD increase in PTP above respective individual baseline means is shown in Table 2. There was considerable variability in the concentration of antigen and number of puffs resulting in an increase of at least 1 SD above a subject’s individual baseline subglottic pressures.

Experimental results

Individual average PTP data from the experimental days are shown in Figure 1. Baseline-normalized individual data are shown in Figure 2. Both figures show that for the placebo day, subglottic pressures were relatively constant across baseline and postplacebo time points. The exception was subject 2. At the 60-minute (but not the 15-minute) follow-up, that subject showed an increase of slightly more than 1 SD above baseline PTP for that day.

Findings for the antigen day were quite different. At the 15-minute postexposure time point, 2 of 5 subjects demonstrated PTPs 1 SD or more above baseline (results ranged from 0.49 to 1.85 SD). However, by 60 minutes, all 5 subjects showed an increase in PTP values from baseline, ranging from 1.07 to 4.25 SD.

Figure 3 displays changes in Z-scores from baseline PTP at 15-minute and 60-minute follow up, collapsed across all subjects. Although the time point was not of central interest in the present study, numerically normalized PTPs appeared to increase considerably more at the 60-minute time point than at the 15-minute time point for the antigen compared to placebo condition.

The repeated measures ANOVA showed a trend—failing to achieve statistical significance—toward a main effect of time (15 vs 60 minutes) on PTP, collapsing across treatments (*p* = 0.071). However, the main effect of treatment (placebo vs allergen), the primary interest in this study, was statistically robust (*p* = 0.013). Despite the appearance of an interaction of time and treatment in Figure 3, the ANOVA did not confirm a significant interaction (*p* = 0.126).

Discussion

The study’s purpose was to determine if vocal function changes measured by PTP would occur following allergen

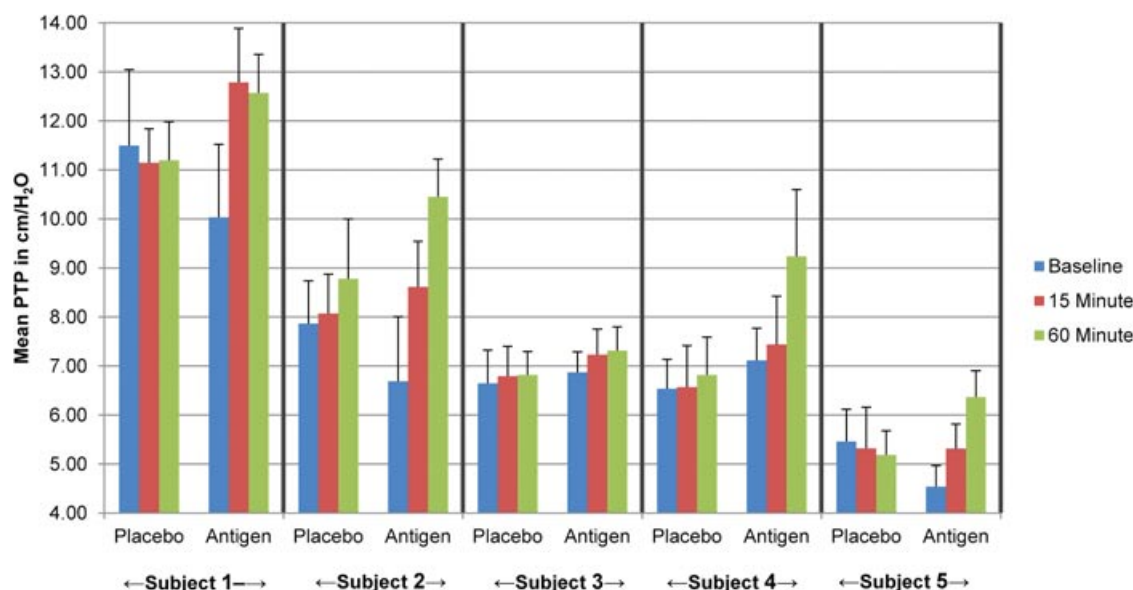


FIGURE 1. Raw data collected on the placebo and antigen experimental days showing the subglottic pressure required to initiate softest phonation in cm/H₂O (PTP) at the subjects 80th percentile high pitch before and following inhalation challenge. Each constellation of 3 bars represents the prechallenge baseline, and 15-minute and 60-minute postchallenge time points for that day. PTP = phonatory threshold pressure.

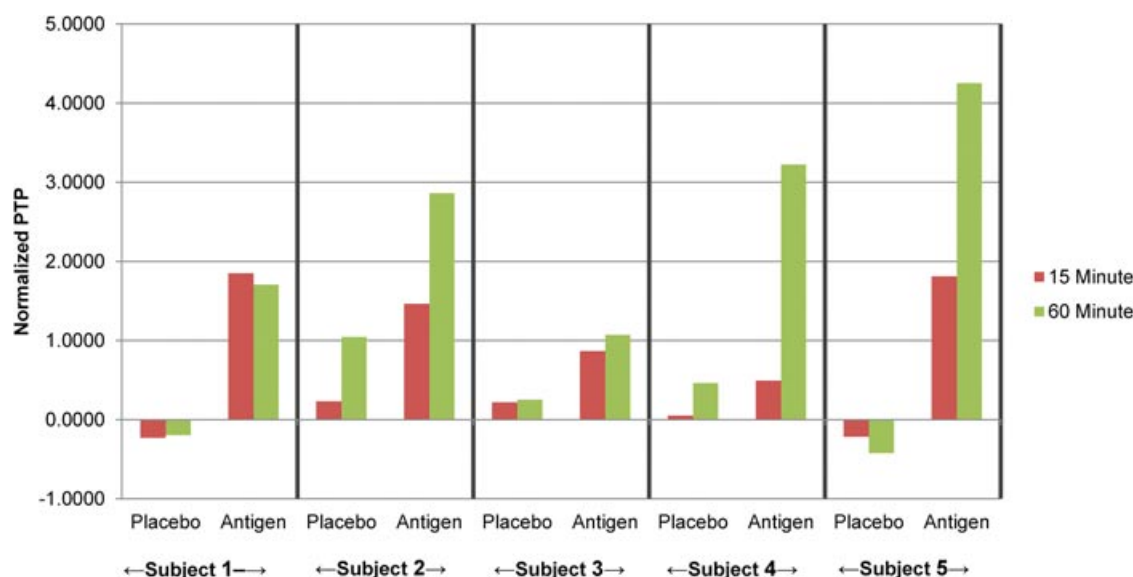


FIGURE 2. Normalized PTP data collected on the placebo and antigen experimental days showing the PTP change in standard deviations for the 2 postchallenge time points compared to the prechallenge baseline. As per the formula used to generate the normalized Z-scores, the prechallenge baseline for each subject becomes zero. PTP = phonatory threshold pressure.

challenge to the larynx for susceptible subjects. The results show that after controlling for subject type by excluding those with reactive airway disease,³² identifying a subject-specific antigen dose, addressing safety issues, and using a theoretically motivated outcome measure, there is a negative impact of allergy on vocal function as captured by high-pitched PTP following allergen exposure compared to placebo exposure. Although the signs and symptoms of laryngeal allergy overlap dramatically with those of LPR,^{5,45} the acute changes seen in the results are unlikely to be related to LPR as the significant vocal changes observed for all 5 subjects occurred in the hour following allergen

but not placebo challenge. It is highly unlikely that all 5 subjects experienced an LPR event resulting in significant voice changes during the postchallenge hour of the antigen challenge day but not on the placebo challenge day. Nor can these vocal changes be attributed to a reaction in the nose or sinuses, as the antigen was delivered by mouth while subjects wore a nose clip thus eliminating nasal exposure to the antigen. Moreover, results were consistent across subjects. All subjects showed greater PTP increases following antigen compared to placebo exposure.

Although the time course of the vocal response was not a focus in our study design, numerically (but not

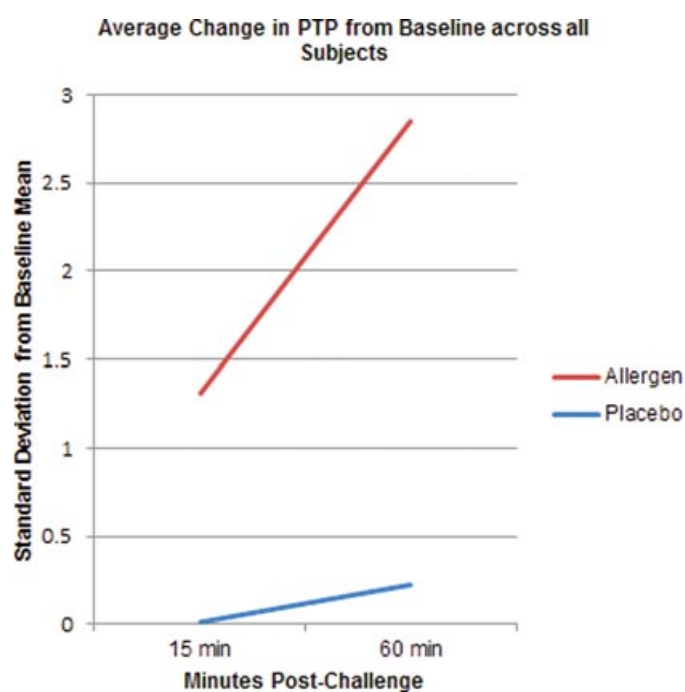


FIGURE 3. Graph shows the PTP change in standard deviations across all subjects for the 2 postchallenge time points compared to the prechallenge baseline for the antigen and placebo experimental days. Notice that the detrimental effects of the allergic reaction on vocal function increased from the 15 minute to 60 minute post-antigen challenge time points. The lack of a maximal response within 15 minutes could explain why previous studies, which have only taken vocal measures within 15 minutes postchallenge, have been unable to capture any vocal changes. PTP = phonatory threshold pressure.

statistically), PTP changes were greater at 60 minutes compared to 15 minutes following antigen exposure. In contrast, nasal symptoms are reported to peak around 10 to 20 minutes following allergen exposure and persist for up to 90 minutes before late phase effects occur at around 5 to 9 hours later.^{46,47} We did not see a statistical difference in time-graded vocal response in our study. Speculatively, if such gradation is confirmed in future, larger-N studies, this time discrepancy compared to the nasal allergen challenge model could be related to several factors, including disparate distribution and concentration of mast cells of nose vs larynx.^{33,48–51} It is possible that the relative lack of mast cells in the vocal fold tissue compared to surrounding laryngeal tissues could result in a delay in vocal changes from allergen exposure because inflammatory mediators from the surrounding tissues must diffuse into the vocal folds to cause physical changes and thus affect vocal function. Again, this is speculative and there is currently no statistical evidence suggesting laryngeal changes are more pronounced at the later time period.

These data provide the first placebo-controlled, double-blind evidence that impaired vocal functioning can be caused by a transoral allergen challenge to the larynx in susceptible individuals while controlling for asthma and processes originating from the nose. To our knowledge, this is the first study demonstrating an allergic response af-

fecting vocal performance in a placebo-controlled, double-blind design. Additional work assessing the mechanisms behind the responses would be possible using a model similar to one designed for this study. For the vocal changes to be clearly linked to a type I, immunoglobulin E (IgE)-mediated allergic response within the larynx, a study showing evidence of mast cell degranulation is needed. In addition, serial sampling of allergic mediators from the larynx might elucidate the pathophysiologic basis of a possible delay of the laryngeal response to allergy to 60 minutes rather than 15 minutes.

It is possible the changes in vocal function could be attributed to the allergen acting as a general laryngeal irritant, which might also occur in nonallergic individuals, even in the context of a negative response to a diluent placebo control. However, we think this unlikely based on the observations from 1 subject (subject 6) who had no response to the highest concentration of nebulized cat antigen during the dose establishment day even though she had previously tested positive to cat allergen. We subsequently discovered she had been treated with immunotherapy for cat and had discontinued allergy shots over 1 year before her participation in the study. Post hoc intradermal testing showed she no longer had an allergy to cat. If the increase in PTP after antigen exposure in the other 5 subjects was not allergy-related but was rather a response to a nonallergenic irritant, then we would have expected this sixth subject to have had a similar increase in PTP. However, she did not. It is also possible, extrapolating from this subject's experience, that allergen desensitization may be an effective treatment for allergy-related voice complaints. Further study specifically directed to this treatment aspect is required.

This study is the only one to date to evaluate laryngeal performance responses up to a 60-minute postexposure time point. Interestingly, although responses varied at the 15-minute postexposure time point in our study, all 5 subjects showed a pronounced worsening in vocal function at the 60-minute postexposure assessment compared to baseline. This factor may explain why previous studies assessing outcomes only shortly after provocation may have failed to identify evidence of laryngeal allergic dysphonia.

Limitations of our study include the small number of participants. However, the effect size for the allergen vs placebo effect was nontrivial (0.821), and statistical significance emerged despite the small N, implying the findings are likely to be robust. While it is impossible to exclude allergen stimulation of other portions of the airway using this model with absolute certainty, any potential stimulation was minimized by the use of oral inhalation, a nose clip, and exclusion of methacholine responsive subjects.

LPR can mimic many of the symptoms of "allergic laryngitis"^{5,45} and we did not exclude patients with LPR. While there are studies and screening tools that can be used to assist in the diagnosis of LPR,^{52–55} and a new impedance-pH catheter that allows detection of the proximal hypopharyngeal reflux events associated with LPR,⁵⁶ up until our study, there has been no method to diagnose

“allergic laryngitis.” The histologic underpinnings of “allergic laryngitis” are based on recent animal models showing mast cell degranulation in supraglottic areas, with allergen provocation⁵⁷ and histologic studies of animal^{48,50} and human laryngeal mucosa.^{33,49} Whether this mast cell laryngeal degranulation is from direct allergen stimulation or from effects of systemic allergen exposure are unknown. In humans, allergic stimulation in 1 portion of the airway can produce eosinophilia in another unstimulated portion. This has been shown with bronchial allergen provocation producing nasal eosinophilia and conversely, nasal allergen challenge producing bronchial eosinophilia.^{58–64} To isolate any vocal changes resulting from antigen exposure to a primary allergic response in the larynx, our method attempted to (1) minimize the stimulation of the sinonasal area with the use of oral inhalation utilizing a nose clip, and (2) minimize the effects of bronchial reactivity on voice by excluding subjects responsive to methacholine challenge.

Conclusion

The current study establishes a safe laryngeal allergic model, which (1) minimizes the danger of provoking reactive airway disease, (2) minimizes potential confounders associated with contamination by nasal allergen exposure or asthma, (3) achieves titration of an allergen dose which is both safe and provocative, and (4) shows that laryn-

geal allergen challenge in susceptible individuals provokes vocal dysfunction indicating that a primary allergic response in the larynx does exist. Forty-five patients were screened to enroll the 5 who completed the study, highlighting the rigor required in this model to show laryngeal allergen effect in a safe manner. However, 1 of the inclusion criteria stipulated that subjects had to present with a priori vocal complaints associated with allergy. Given the large effect size and the subjective inaudibility of the voice changes for most subjects, it is likely that measurable voice changes would be found even in individuals unaware of voice changes related to allergy. Eliminating this inclusionary criterion will greatly increase subject recruitment. In the future, we would make sure that PTP at 60 minutes was a primary endpoint, as it was more reliably positive than assessment at 15 minutes. Conclusive proof of IgE-mediated mechanism of laryngeal allergy would be further supported with evidence of laryngeal mast cell degranulation or interventions blocking allergen induced voice changes specific to blocking allergic effects. The current study provides the first placebo-controlled double-blind evidence for primary laryngeal inhalant allergic responses. Future studies examining laryngeal cytokine changes with laryngeal allergy stimulation and response to allergy treatment intervention will advance our understanding of the pathophysiology of allergen-induced vocal complaints and dysphonia. ④

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