



Clinical symptoms and biomarkers of Bermuda grass–induced allergic rhinitis using the nasal allergen challenge model

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ARTICLE INFO

Article history:

Received for publication November 20, 2019.

Received in revised form March 3, 2020.

Accepted for publication March 5, 2020.

ABSTRACT

Background: Bermuda grass is a prevalent allergen that flourishes in tropical climates. Its exposure is traditionally believed to be low in Ontario due to the colder environment. However, high sensitization rates have been observed in Kingston, Ontario.

Objective: We sought to investigate whether its allergens can provoke allergic rhinitis (AR) symptoms in sensitized participants from south-eastern Ontario and determine if nasal allergen challenge (NAC) model is appropriate to study Bermuda grass–induced AR.

Methods: Twenty-one participants sensitized to Bermuda grass and 12 nonallergic participants completed a titrated NAC with increasing allergen concentrations at a screening visit. Total nasal symptom score (TNSS) and peak nasal inspiratory flow were collected before allergen exposure and 10 minutes after delivery of each concentration. Twelve participants with a Bermuda grass allergy who met the qualifying criteria (TNSS ≥ 8 and peak nasal inspiratory flow fall $\geq 50\%$) and 11 nonallergic controls returned for single-dose NAC visit.

Results: At titrated NAC, 19 of 21 sensitized participants met the criteria of positive allergic response when challenged. During single-dose NAC, participants with allergy had significantly greater TNSS between 15 minutes and 3 hours after NAC than controls. Likewise, allergic participants had a significantly increased number of nasal lavage eosinophils at both 1 and 6 hours after NAC. Bermuda grass–specific immunoglobulin E was significantly increased in Bermuda grass allergic participants at NAC than screening visit.

Conclusion: Although Bermuda grass is a non-native allergen in Ontario, it can induce AR symptoms in sensitized participants, and the NAC model is appropriate to study Bermuda grass–induced AR.

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Disclosures: Dr Ellis has participated in advisory boards for ALK Abello, Bausch Health, Circassia Ltd, GlaxoSmithKline, Johnson & Johnson, Kaleo, Merck, Novartis, PEDIAPHARM, Pfizer, and Sanofi; she has been a speaker for ALK Abello, AstraZeneca, Boehringer Ingelheim, Mylan, Merck, Novartis, Nuvo, PEDIAPHARM, Pfizer, and Takeda. Her institution has received research grants from Bayer LLC, Circassia Ltd, Green Cross Pharmaceuticals, GlaxoSmithKline, Sanofi, Sun Pharma, Regeneron, Merck, Novartis, and Pfizer. She is also a consultant to Bayer LLC and Regeneron. Mr Rawls is a current employee of STEMCELL Technologies. All the remaining authors have no conflicts of interest to report.

Funding Sources: No specific grants were received from funding agencies in the public, commercial, or not-for-profit sectors.

<https://doi.org/10.1016/j.anai.2020.03.003>

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Introduction

Allergic rhinitis (AR) is an immunoglobulin E (IgE)–mediated inflammatory disorder of the nose. The estimated AR prevalence ranges from 11.9% to 30.2% in the United States¹ and 20% to 25% in Canada.² In North America, grass pollens are common allergens that can provoke AR symptoms. The 2005–2006 US National Health and Nutrition Examination Survey reported a positive IgE-specific sensitization for perennial rye grass and Bermuda grass pollens in 19.5% and 15% of participants, respectively.³ It is also estimated that the prevalence of grass sensitivity ranges from 14% to 30% in Canadian patients with AR.⁴ Typical symptoms of AR include sneezing, nasal itching, congestion, and postnasal drip. Uncontrolled AR symptoms can have a negative impact on sleep quality, work productivity, driving ability, and academic performance.^{5–13} These symptoms may be addressed by a variety of different treatment options, such as antihistamines and nasal corticosteroids, to

reduce their severity. The only therapeutic option that addresses the underlying etiology of AR is allergen immunotherapy (AIT). Despite the available antiallergic medications, approximately 60% of patients with AR still experience lifestyle limitations.²

Experimental AR models were developed to diagnose, study the pathophysiology, and investigate antiallergic medications. Controlled nasal challenges (CNCs), commonly known as nasal allergen challenges (NACs) or nasal provocation tests, are the gold standard to diagnose AR.^{14–17} CNCs involve direct exposure of diluted allergen extract into the nasal cavity in a standardized manner. CNCs are helpful for physicians when diagnosing AR, especially in patients with unclear history of allergen exposure. There are numerous techniques available to evaluate AR symptoms during CNCs. The Total Nasal Symptom Score (TNSS) is a Food and Drug Administration (FDA)–approved self-reported questionnaire to evaluate AR nasal symptoms.¹⁸ An objective measure that can be used during CNCs is peak nasal inspiratory flow (PNIF). PNIF is a cost-efficient tool that measures nasal patency in participants of AR.^{19–21} In Ontario, the Kingston Allergy Research group has investigated a wide variety of allergens utilizing an optimized and standardized NAC model.^{21–26} However, the group has yet to investigate nonlocal environmental allergens using this same NAC model.

One non-native allergen of interest has been Bermuda grass pollen. Bermuda grass (*Cynodon dactylon*) is a common lawn grass that thrives in both tropical and subtropical environments.²⁷ Bermuda grass pollen is an extremely prevalent aeroallergen in southern United States. Exposure to Bermuda grass in Ontario is traditionally believed to be relatively low due to the colder environmental conditions.^{28,29} Despite the limited routes of Bermuda grass pollen exposure, the Kingston Allergy Research group has observed a high positive sensitization rate, as determined by the skin prick test, to Bermuda grass extract in Kingston and the surrounding area.³⁰ Likewise, Nottle et al²⁹ reported that 64% of the Canadians screened across multiple centers were sensitized to Bermuda grass with an average Bermuda grass–specific IgE (sIgE) of 7.83 kU/L. It is currently unclear how Canadians develop Bermuda grass sensitization. One possible explanation for this sensitization involve previous residence or visitation of regions where Bermuda grass pollen is abundant. Alternatively, sensitization may be through a cross-reactive mechanism to local grass pollens. However, sensitization alone does not guarantee that a patient will respond with AR symptoms when exposed to Bermuda grass pollen. Therefore, this study aimed to investigate whether Bermuda grass allergen extract can provoke AR symptoms in Canadian participants sensitized to Bermuda grass utilizing the Allergic Rhinitis–Clinical Investigator Collaborative (AR-CIC) NAC model.

Methods

The Bermuda Grass Cumulative Allergen Challenge (BerCAC) study was designed to investigate and study Bermuda grass–induced AR in humans using CNC. Participants were recruited from Kingston, Ontario, and the surrounding area. This study received ethical clearance from the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board. All study participants gave informed written consent before partaking in the study. The BerCAC study was performed outside the native grass pollen season.

Inclusion and Exclusion Criteria

Participants were recruited based on the documentation of Bermuda grass sensitization. Sensitized participants, as defined by a positive Bermuda grass skin prick test (wheal diameter ≥ 3 mm above negative control of extract diluent), were included.

Sensitized participants were also tested to a panel of common aeroallergens (mold, ragweed, grasses, birch, local tree mix, house dust mite, cockroach, cat pelt, and dog hair) using the skin prick test. Nonallergic participants were recruited and included, provided they tested negative for skin prick test to a panel of common aeroallergens, including Bermuda grass extract.

Participants were excluded from the BerCAC study if they were actively trying to conceive, were pregnant, or lactating; had clinically significant nasal structural defects or severe asthma requiring high-dose corticosteroids or a biologic therapy; were being treated with a grass AIT; and expressed active signs and symptoms of AR, a history of respiratory tract infection within the past 2 weeks, or a TNSS greater than 2 before any allergen challenge.

Study Participants

Participants sensitized to Bermuda grass and nonallergic individuals (aged 18–65 years) were recruited from Kingston, Ontario, and the surrounding region. Twenty-one Bermuda grass–sensitized patients and 12 nonallergic participants completed the titrated allergen challenge at a screening visit. Twelve Bermuda grass allergic and 11 participants without allergy completed the NAC visit. All participants were instructed to document their medications and supplements taken 30 days before screening and throughout the study. Antihistamines, corticosteroids, and other antiallergic medications were restricted for various time periods before each study visit (eTables 1 and 2).

Study Design

The AR-CIC NAC model consisted of the following 2 visits separated by approximately 21 to 28 days: screening visit and cumulative single-dose NAC visit (Fig 1). At the screening visit, participants were invited to partake in a titrated allergen challenge. Each participant first received a wash of nasal cavity with sterile saline and then recorded their baseline TNSS and PNIF. Next, a placebo challenge with extract diluent (Stallergenes Greer, Lenoir, North Carolina) was administered to both nostrils using the Pfeiffer Bidose Nasal Delivery Device (Aptar Pharma, Congers, New York). TNSS and PNIF were recorded 10 minutes after placebo challenge. Participants sensitized to Bermuda grass who did not have hypersensitive response received a series of increasing concentrations of Bermuda grass pollen extract ranging from 1:128 to 1:2 (Stallergenes Greer). Each allergen dilution was administered at a volume of 100 μ L per nostril using the Pfeiffer Bidose Nasal Delivery Device. TNSS and PNIF were measured 10 minutes after delivery of each dilution allergen. When the qualifying criteria (TNSS ≥ 8 and PNIF fall $\geq 50\%$) were reached, the titrated challenge was stopped, and the last dilution of Bermuda grass extract received was classified as the qualifying concentration. All nonallergic controls received a 1:2 dilution of Bermuda grass pollen extract (Stallergenes Greer) after the placebo challenge.

Study participants who met the qualifying criteria were invited to return for NAC visit 21 to 28 days after screening. In contrast to the screening visit, a single, personalized, cumulative allergen dilution (sum of all allergen dilutions received at screening) of Bermuda grass pollen extract was delivered to both nostrils. After NAC, TNSS and PNIF were recorded incrementally at 15 minutes, 30 minutes, 1 hour, and hourly until 12 hours after the challenge. Additional time point for symptoms was collected 24 hours after NAC.

Symptom Diary Cards

Symptoms of postnasal drip, nasal congestion and/or stuffiness, nasal itching, sneezing, itchy and/or watery eyes, red and/or burning eyes, and itchy ears, palate and/or throat were self-rated by each participant on 4-point scale (0–3). Complete absence of symptoms was recorded as “0.” Mild symptom that was not

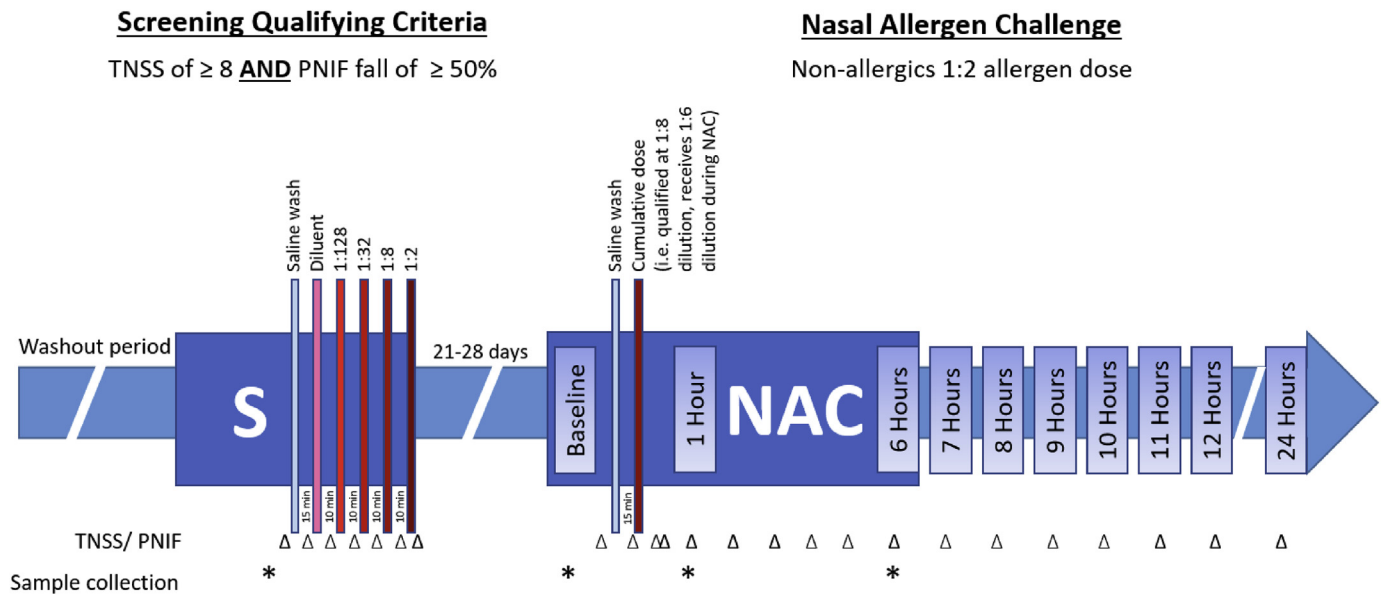


Figure 1. The nasal allergen challenge (NAC) experimental design. The screening visit (S) involved a titrated allergen challenge where the qualifying allergen dilution was determined using incremental concentrations of Bermuda grass allergen until Bermuda grass-sensitized participants reached the qualifying criteria (total nasal symptom score (TNSS) of $\geq 8/12$ and peak nasal inspiratory flow (PNIF) fall of $\geq 50\%$). During the NAC visit, participants were challenged with a single cumulative allergen dilution equivalent to the sum of each dilution they received during screening. Nonatopics received the greatest allergen concentration (1:2).

bothersome was documented as “1.” Score of “2” was described as moderate symptom that was tolerable but bothersome. Severe and difficult-to-tolerate symptom that a participant desired treatment for was classified as “3.” TNSS, Total Ocular Symptom Score (TOSS), and Total Rhinoconjunctivital Symptom Score (TRSS) were calculated and reported. PNIF was also used to measure nasal airflow and nasal obstruction for each participant using an In-Check PNIF meter (Clement Clarke International Ltd Harlow, United Kingdom).

Blood Collection and Processing

Venous peripheral blood was collected from each participant at the screening visit and during NAC visit at baseline, 1 hour, and 6 hours after NAC. Peripheral blood was allowed to clot for a minimum of 1 hour at room temperature and then centrifuged at 1300g for 10 minutes. Bermuda grass sIgE was measured using a Phadia 100 system (Thermo Fisher Scientific, Waltham, Massachusetts) and Bermuda grass ImmunoCAP assays (Somagen Diagnostics Inc, Edmonton, Alberta, Canada).

Nasal Lavage Collection and Processing

Nasal lavage samples were collected from all participants during the NAC visit at baseline, 1 hour, and 6 hours after challenge. Participants were instructed to sit in a forward flexed neck position. Using a sterile syringe and an appropriately sized sterile nasal olive, 5.0 mL of 0.9% sterile saline was gently flushed into the nostril for approximately 1 minute. The fluid was collected into a 15-mL polystyrene tube for each participant and placed directly on ice. Nasal lavage tubes were centrifuged at 600g for 4 minutes. The cellular pellet was resuspended in Dulbecco's phosphate-buffered saline with 0.05% Tween 20 and counted with Trypan blue to obtain a total cell count. Nasal lavage samples were diluted to reach a concentration of 200,000 cells/mL. Shandon Cytospin 4 (Thermo Fisher Scientific) and cytofunnels were used to spin 100 μ L of resuspended cell solution onto each slide. Cells from the nasal lavage were differentially stained using a Diff-Quick staining set (Siemens, Munich, Germany) and counted using a light microscope by at least 2 trained laboratory members. Neutrophils, eosinophils,

macrophages, lymphocytes, and epithelial cells were counted and recorded until reaching a minimum of 250 cells per slide.

Statistics

GraphPad Prism 8.1.2 was used to perform all statistical tests and generate all figures. Mann-Whitney *U* tests were used to compare mean peak TNSS, percent PNIF fall, and wheal size between Bermuda grass-sensitized participants and healthy controls. Two-way analysis of variance (ANOVA) tests with Bonferroni's multiple comparison tests were used to compare the mean TNSS, TRSS, TOSS, PNIF, and change in PNIF between Bermuda grass allergic and nonallergic participants during the NAC visit. Kruskal-Wallis tests with Dunn's multiple comparison tests were utilized to investigate progression of AR clinical symptoms in participants with allergy during the NAC visit. The progression of PNIF and change in PNIF during the NAC visit for the participants with allergy were analyzed using a repeated measures one-way ANOVA with Dunnett's test and Friedman test with Dunn's multiple comparisons test, respectively. Wilcoxon rank tests were also used to investigate the difference in mean serum Bermuda grass sIgE for participants with allergy at screening than NAC visit. Mann-Whitney *U* tests and Friedman tests with Dunn's multiple comparisons tests were used to calculate the statistical significance of the nasal lavage data.

Results

Screening Visit Results

Twenty-one Bermuda grass-sensitized participants and 12 nonallergic controls completed a screening titrated allergen challenge with Bermuda grass pollen extract. Bermuda grass wheal size ($P < .0001$, Fig 2A) and peak TNSS ($P < .0001$, Fig 2B) were both significantly greater in sensitized participants than the controls. PNIF fall for sensitized participants was significantly greater than controls without allergy ($P < .0001$, Fig 2C). Based on the European Academy of Allergy and Clinical Immunology guidelines,¹⁴ 19 of 21 sensitized participants achieved a clearly positive allergic response by reaching TNSS of at least 5 when challenged. Fifteen sensitized

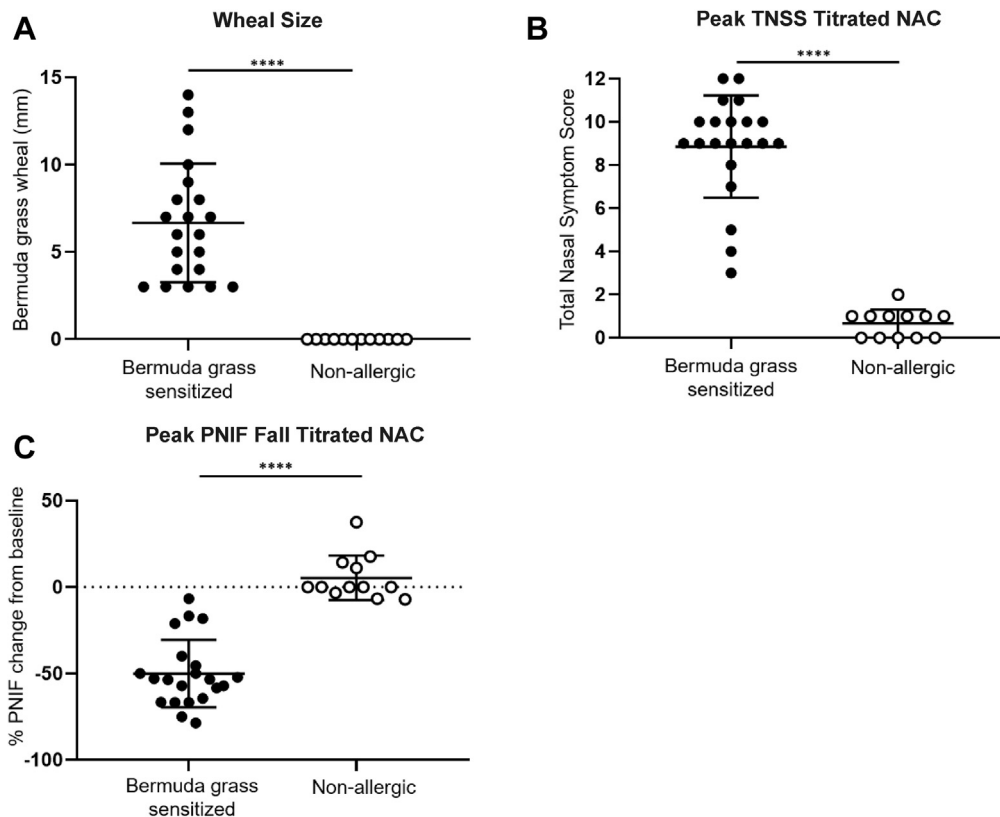


Figure 2. Bermuda grass-sensitized participants expressed symptoms consistent with those with allergic rhinitis during titrated allergen challenge. The Bermuda grass wheal size data (A) for sensitized ($n = 21$) and without allergy ($n = 12$) participants were collected during the screening visit. Mean wheal size was significantly greater in Bermuda grass-sensitized participants than nonallergic controls ($P < .0001$, Mann-Whitney U test). Bermuda grass-sensitized participants also had significantly elevated peak total nasal symptom score (TNSS) than nonallergic participants ($P < .0001$, Mann-Whitney U test) (B). Likewise, the percent peak nasal inspiratory flow (PNIF) fall was significantly greater in Bermuda grass-sensitized participants than in nonallergic controls ($P < .0001$, Mann-Whitney U test) (C). NAC, nasal allergen challenge.

participants met the predetermined qualifying criteria for the BerCAC study (TNSS ≥ 8 and PNIF fall $\geq 50\%$). All Bermuda grass-sensitized participants who completed the screening visit also had positive skin prick test result to Timothy grass pollen extract.

Demographics of Qualifying Participants for the NAC Visit

Twelve Bermuda grass allergic and 11 nonallergic participants returned to successfully complete the NAC visit (Table 1). There were no significant differences between participants with and without allergy regarding sex, mean age, height, and weight. The mean wheal size of the 12 Bermuda grass allergic participants who returned was significantly greater than the nonallergic controls (7.8 mm, SD = 3.19 vs 0.0 mm, SD = 0.0, $P < .0001$).

Comparison of Clinical Symptoms of Allergic and Nonallergic Participants

The mean TNSS, TRSS, and TOSS were plotted for both participants during the NAC visit (Fig 3). The mean TNSS was significantly greater in participants with Bermuda grass allergy than nonallergic controls at 15 minutes ($P < .0001$), 30 minutes ($P < .0001$), 1 hour ($P < .0001$), 2 hours ($P < .0001$), and 3 hours ($P = .0004$) after the allergen challenge. Likewise, TRSS was significantly elevated after NAC when comparing Bermuda grass allergic to nonallergic participants (15 minutes, $P < .0001$; 30 minutes, $P < .0001$; 1 hour, $P < .0001$; 2 hours, $P < .0001$; 3 hours, $P = .0016$; 4 hours, $P = .0465$). In addition, the mean TOSS was significantly greater in participants with Bermuda grass allergy than controls at 15 minutes ($P < .0001$), 30 minutes ($P < .0001$), 1 hour ($P = .0017$), and 2 hours ($P = .0067$)

after the allergen challenge. The mean PNIF achieved statistical significance when comparing allergic participants to nonallergic controls only at 30 minutes ($P = .0035$) after NAC (Fig 4). The mean PNIF fall was significantly greater during the 15 minute ($P = .0201$) and 30 minute ($P = .0003$) time points in the allergic participants compared to nonallergic controls.

Progression of Clinical Symptoms in Allergic Participants During NAC

Bermuda grass allergic participants expressed a peak in reported symptoms approximately 15 to 30 minutes after the challenge (eFig 1). They had a significant increase from baseline in

Table 1
BerCAC Study Demographics

Characteristics	Bermuda grass allergic (n = 12)	Nonallergic (n = 11)
Mean age, y (SD)	45.8 (12.0)	40.9 (14.7)
Women, n (%)	8 (66.6)	8 (72.7)
Mean weight, kg (SD)	83.33 (25.09)	81.24 (15.84)
Mean height, cm (SD)	164.4 (7.97)	163.2 (7.43)
Race, n (%)		
White	11 (91.6)	10 (90.9)
White and Asian	1 (8.3)	0 (0)
Southern Asia	0 (0)	1 (9.1)
Mean wheal size, mm (SD)		
Bermuda grass	7.75 (3.19)	0.00 (0)
Timothy grass	11.75 (5.80)	0.00 (0)
Bermuda grass sIgE, kUA/L, (SD)	3.46 (5.78)	0.01 (0.01)

Abbreviation: BerCAC, Bermuda Grass Cumulative Allergen Challenge.

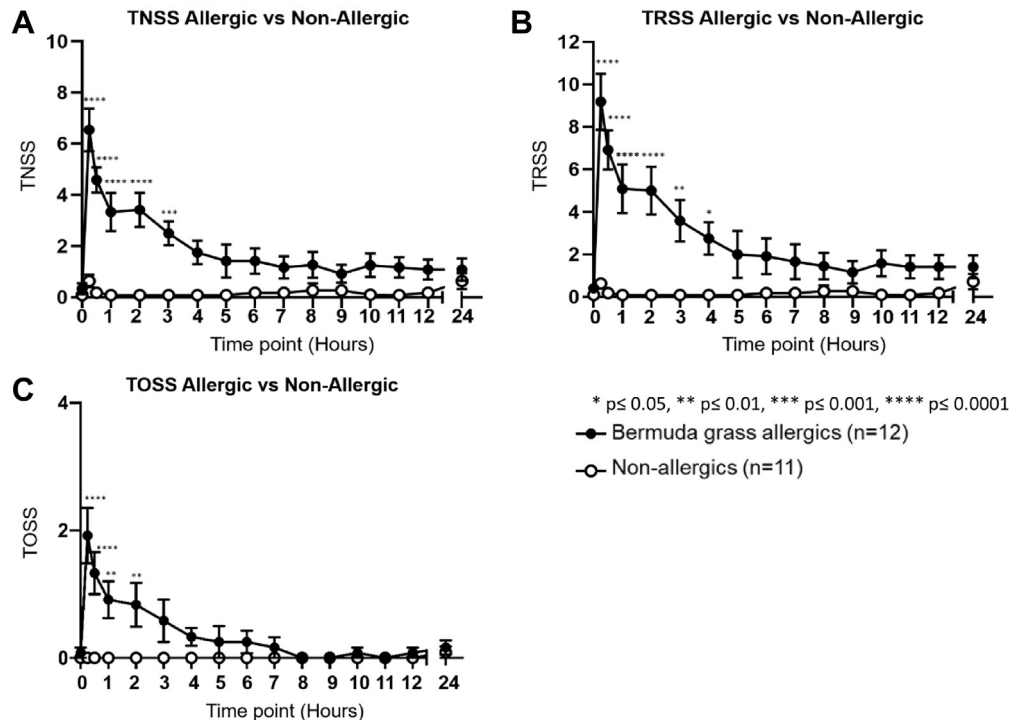


Figure 3. During the nasal allergen challenge visit, allergic rhinitis symptoms were greater in allergic participants than nonallergic controls. Mean total nasal symptom score (TNSS) (A), total rhinoconjunctivitis symptom score (TRSS) (B), and total ocular symptom score (TOSS) (C) were plotted for the participants with and without allergy. Mean TNSS was significantly greater at 15 minutes ($P < .0001$), 30 minutes ($P < .0001$), 1 hour ($P < .0001$), 2 hours ($P < .0001$), and 3 hours ($P = .0004$) after challenge when comparing allergic participants to nonallergic controls. Likewise, mean TRSS was significantly higher for allergic participants at 15 minutes ($P < .0001$), 30 minutes ($P < .0001$), 1 hour ($P < .0001$), 2 hours ($P < .0001$), 3 hours ($P = .0016$), and 4 hours ($P = .0465$) after challenge than nonallergic controls. For participants with Bermuda grass allergy, the mean TOSS was significantly greater at 15 minutes ($P < .0001$), 30 minutes ($P < .0001$), 1 hour ($P = .0017$), and 2 hours ($P = .0067$) after allergen challenge than the healthy controls (two-way ANOVAs with Bonferroni's tests).

mean TNSS at 15 minutes ($P < .0001$), 30 minutes ($P < .0001$), 1 hour ($P = .0042$), 2 hours ($P = .0013$), and 3 hours ($P = .0173$) after NAC. Likewise, their mean TRSS was significantly elevated (15 minutes, $P < .0001$; 30 minutes, $P < .0001$; 1 hour, $P = .0022$; 2 hours, $P = .0009$; 3 hours, $P = .0209$). Their TOSS was significantly increased compared with baseline at 15 minutes after NAC ($P = .0168$). Their PNIF was significantly decreased 15 minutes ($P = .0007$), 30 minutes ($P = .0005$), and 1 hour ($P = .0187$) after NAC compared with

baseline (eFig 2). They also had a significant decrease in percent PNIF fall for 2 hours after NAC (15 minutes, $P < .0001$; 30 minutes, $P < .0001$; 1 hour, $P = .0011$; and 2 hours, $P = .0464$).

AR Phenotypes

A total of 10 Bermuda grass allergic participants were characterized by previously set guidelines for phenotyping.²¹ Two allergic

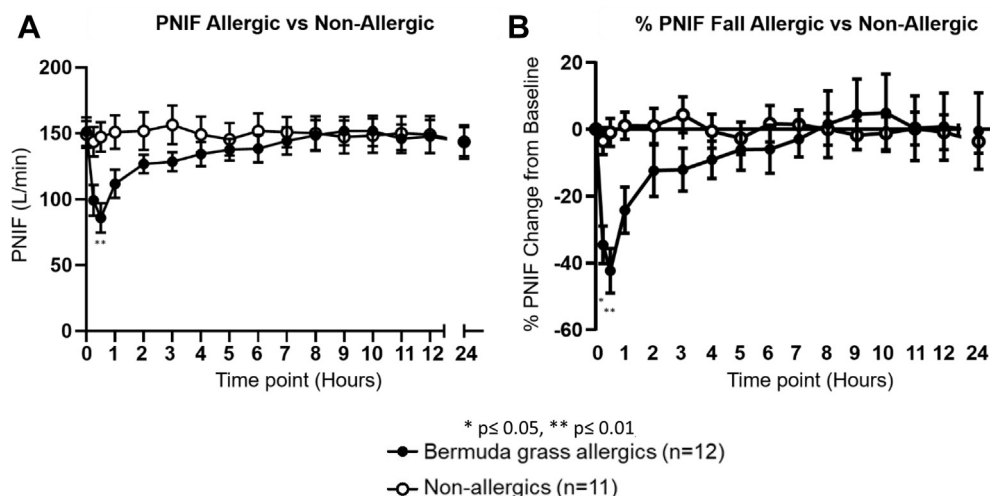


Figure 4. Peak nasal inspiratory flow (PNIF) was significantly reduced during nasal allergen challenge in Bermuda grass allergic participants compared to nonallergic controls. The mean and standard error of the mean for PNIF (A) and percent PNIF fall (B) were plotted for both allergic and nonallergic participants. Mean PNIF was significantly lower at 30 minutes after challenge in allergic participants compared to nonatopic controls ($P = .0035$). The mean PNIF fall was significantly greater at 15 minutes ($P = .0201$) and 30 minutes ($P = .0003$) for allergic participants after nasal allergen challenge than the nonallergic controls (two-way ANOVAs with Bonferroni's tests).

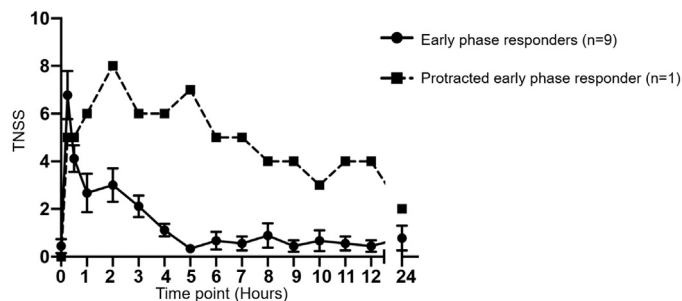


Figure 5. Total Nasal Symptom Score (TNSS) from the nasal allergen challenge visit were examined to identify each allergic participant's phenotypic response. Allergic participants were classified into 2 phenotypic groups: early-phase responders (EPRs) and protracted early-phase responders (pEPRs). EPRs had a quick response to allergen exposure with a peak in allergic rhinitis symptoms between 15 and 30 minutes, followed by a gradual reduction toward baseline by the 5th to 7th hour after the challenge. A total of 90% of participants with allergy were categorized as EPRs. The pEPR had a quick rise in nasal symptoms, and symptoms remained elevated above baseline until 24 hours after nasal allergen challenge. Only 1 participant with allergy was identified as a pEPR. No dual-phase responder was recognized during the Bermuda Grass Cumulative Allergen Challenge Study. No statistical testing was performed to compare phenotypic groups owing to the low sample size in the pEPR group.

participants were excluded from phenotyping due to incomplete TNSS datasets. Nine early-phase responders (EPRs) were identified in the BerCAC study (Fig 5). EPRs had an initial strong reaction followed by decrease of at least 50% in TNSS by the 6th or 7th hour. In addition, EPRs did not experience any additional sustained increases in TNSS but instead returned to baseline by the 12th hour. Similar to EPRs, protracted early-phase responders (pEPRs) had an initial increase in nasal symptoms. However, pEPRs neither experienced a decrease of at least 50% in TNSS nor returned to baseline by the 12th hour. One participant in the BerCAC study was classified as a pEPR. No dual-phase responders (DPRs) were observed during the NAC study. DPRs experience similar reactions to EPRs but instead had a sustained increase after the 6th or 7th hour.

Bermuda Grass sIgE During the BerCAC Study

At screening, participants with a Bermuda grass allergy had mean serum Bermuda grass sIgE concentration of 3.92 kUA/L ($n = 15$, $SD = 6.27$, Fig 6A). Nonallergic participants had mean serum Bermuda grass sIgE concentration of 0.01 kUA/L ($SD = 0.01$) during the screening visit. Allergic participants had significantly greater concentration of Bermuda grass sIgE than those without ($P < .0001$).

Allergic participants who completed the BerCAC study expressed a mean Bermuda grass sIgE of 3.46 kUA/L ($n = 12$, $SD = 5.78$), 9.16 kUA/L ($SD = 14.89$), and 8.86 kUA/L ($SD = 15.06$) at screening, baseline NAC, and 6 hours after NAC, respectively (Fig 6B). The observed mean Bermuda grass sIgE was significantly elevated when comparing screening to baseline NAC ($P = .0020$) and 6 hours after NAC ($P = .0063$).

Nasal Eosinophil and Total Cell Counts During NAC

Eosinophil cell counts, as percentage of white blood cells, were visibly increased in nasal lavage samples of Bermuda grass allergic participants compared to nonallergic controls during the NAC visit (Fig 7). Participants with a Bermuda grass allergy had significantly higher eosinophil cell counts than those without at both 1 hour and 6 hours after NAC ($P = .0003$ and $P = .0007$). For allergic participants only, the percentage of eosinophils was significantly increased at both 1 hour and 6 hours after nasal challenge than at

baseline ($P = .0002$ and $P = .0066$). No significant differences were observed in total cell counts from nasal lavage samples.

Discussion

The BerCAC study advanced the AR field by evaluating nasal symptoms with a non-native allergen. In Ontario and across Canada, there have been reports of Bermuda grass sensitization.^{29,30} However, neither skin sensitization nor presence of sIgE alone guarantees that a patient will respond with AR symptoms when exposed. Bermuda grass exposure is traditionally thought to be low in Ontario therefore making it difficult for physicians and allergists to establish a history of nasal symptoms when exposed to Bermuda grass pollen.^{28,29} Furthermore, there is a lack of evidence investigating Canadian Bermuda grass-sensitized participants' allergic response when exposed to Bermuda grass pollen. The BerCAC study demonstrated that sensitized participants expressed AR symptoms when exposed to Bermuda grass pollen extract within a NAC model. According to the European Academy of Allergy and Clinical Immunology guidelines, approximately 90% of Bermuda grass-sensitized participants had clearly positive nasal symptoms consistent with AR when exposed to Bermuda grass pollen extract.¹⁴ Nonallergic participants did not express AR symptoms and had minimal reduction in inspiratory airflow. Overall, this study suggests that sensitized participants in Ontario can experience AR symptoms when exposed to Bermuda grass allergens.

To demonstrate that the AR-CIC NAC model can clearly distinguish allergic and nonallergic populations in south-eastern Ontario, we challenged both groups to Bermuda grass allergens. In the BerCAC study, participants who were allergic to Bermuda grass had significantly greater nasal symptoms and eosinophil counts during the NAC visit than those who were not. In addition, an immediate decrease in nasal airflow and an acute increase in clinical AR symptoms were observed for Bermuda grass allergic participants during NAC. An acute response with nasal symptoms and PNIF has been previously demonstrated as a common pattern for NAC model.^{21,22,24,25} NAC provides an effective method for studying AR and investigating Bermuda grass-induced AR symptoms.

The concentration of serum Bermuda grass sIgE was successfully examined at 3 time points during the BerCAC study. Bermuda grass sIgE was significantly increased at the NAC visit in allergic participants compared to the screening visit. This evidence suggests that exposure to allergen at screening may have stimulated the allergen sIgE production. A recent study also reported a significant increase in sIgE at 2, 4, 6, and 8 weeks after allergen challenge.³¹ Other work has previously suggested that higher levels of sIgE may increase clinical sensitivity.³¹⁻³⁴ These results lead to more questions regarding a possible priming effect. A priming effect refers to an initial increase in the sensitivity of clinical allergic symptoms after repeated allergen exposure.³⁵ It is commonly believed that 4 weeks between allergen challenges is a substantial amount of time to negate the priming effect on clinical symptoms.³⁵ Although priming on clinical symptoms may be resolved 4 weeks after exposure, there could be an underlying biological priming effect resulting in a possible limitation of this study. Future NAC studies, examining sIgE or the impact of medications on sIgE, should consider this observation.

The outcomes of the BerCAC study suggest that Bermuda grass sensitization in Ontario can result in AR symptoms when there is exposure to Bermuda grass pollen. Therefore, sensitized patients traveling to environments rich with Bermuda grass may experience AR symptoms when exposed to Bermuda grass pollen allergens. It is vital that patients with AR are made aware that their symptoms may be provoked in these geographic, warm locations. However, one limitation that was not addressed in this study was the

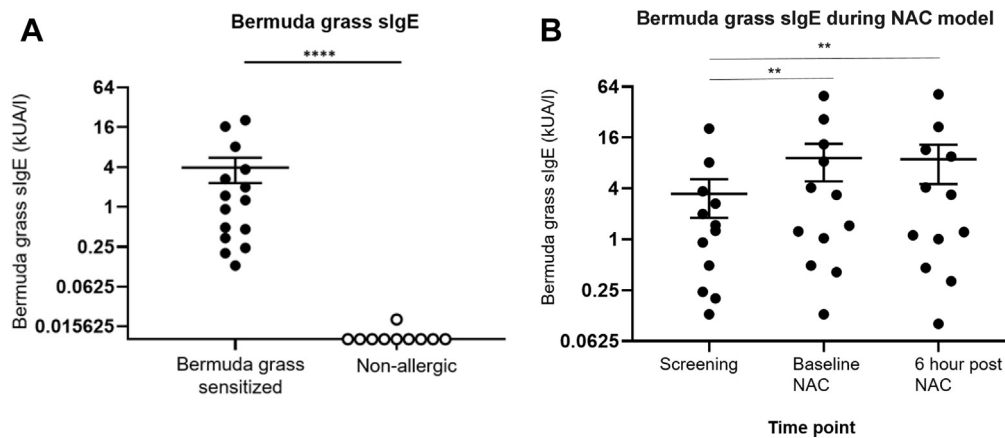


Figure 6. Bermuda grass–specific immunoglobulin E (sIgE) ranged between 0.13 and 20.40 kU/L for the Bermuda grass allergic participants ($n = 15$) who achieved the qualifying criteria at the screening visit (total nasal symptom score ≥ 8 and peak nasal inspiratory flow fall of at least 50%). The mean Bermuda grass sIgE concentration was significantly greater in allergic participants compared to nonallergic controls ($P < .0001$, Mann-Whitney U test) (A). Serum Bermuda grass sIgE for participants with a Bermuda grass allergy were further analyzed at 3 different time points during the Bermuda Grass Cumulative Allergen Challenge Study (B). The mean and standard error of the mean were plotted for the Bermuda grass sIgE levels of allergic participants. The mean Bermuda grass sIgE concentration was significantly increased at baseline (nasal allergen challenge (NAC) visit) and 6 hours after NAC compared with the sample collected at the screening visit ($n = 10$; baseline NAC visit vs screening, $P = .0020$; 6 hours after NAC vs screening, $P = .0063$, Wilcoxon tests).

potential route of sensitization. Bermuda grass pollen concentrations have not been formally investigated in Ontario. Local environmental pollen counts do not differentiate among grass species and instead classify all grass pollens as “true grasses.” The different grass pollen species are commonly grouped together owing to similarities in size, shape, and number of pores when observed under the microscope.

One limitation of the BerCAC study that should be noted was that travel or previous residency histories of participants were not collected. Therefore, it is possible that sensitized participants may have visited regions with Bermuda grass pollen exposure. Although this study did not assess the routes of exposure, a separate study completed by the Kingston Allergy Research group examined previous residence in areas with high Bermuda grass exposure.³⁰ A phone survey was completed with 100 participants who were dual sensitized to both Bermuda grass and

Timothy grass allergens.³⁰ A total of 85% of participants reported not living outside of Canada for more than 1 year.³⁰ However, approximately 20% did live outside of the United States and Canada for a minimum of 3 months.³⁰ A history of vacations or work-related travel to warmer environments was not accounted for during this phone survey.

Recent research has suggested that clinical cross-reactivity between Bermuda grass and Timothy grass pollens may be stronger than traditionally believed.^{36,37} Ramirez et al³⁶ observed that participants with Bermuda grass allergy, who were not previously naturally exposed to Timothy grass pollen, tested positive for AR symptoms when challenged to Timothy grass allergens in a controlled allergen challenge facility. It is possible that the BerCAC study participants may be experiencing a cross-reactivity reaction; however, further investigation is required. Increased evidence supporting the potential cross-reactivity between Bermuda grass

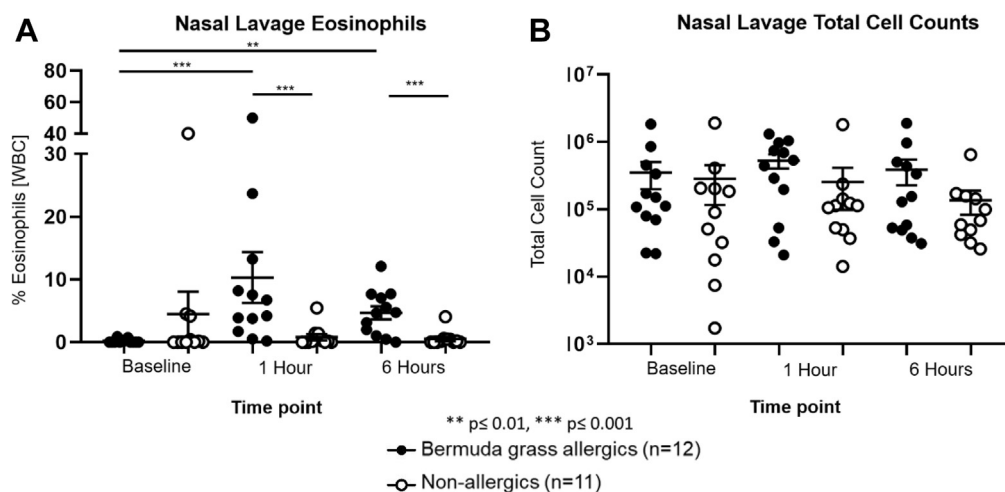


Figure 7. Nasal eosinophil counts (A) were significantly increased after challenge in Bermuda grass allergic participants, whereas total cell counts (B) remained unchanged. The mean eosinophil cell counts were plotted as percentage of white blood cells. Eosinophil cell counts were significantly elevated in nasal lavage samples collected after challenge from allergic participants when compared to nonallergic controls (1 hour, $P = .0003$; 6 hours, $P = .0007$; Mann-Whitney U tests). Likewise, the mean eosinophil cell counts were significantly increased at 1 hour and 6 hours after nasal allergen challenge compared with baseline in allergic participants only (1 hour vs baseline, $P = .0002$; 6 hours vs baseline, $P = .0066$; Friedman test with Dunn's test). No significant differences were observed in the mean total cell counts between the nasal lavage samples of the participants with and without allergy.

and Timothy grass pollens may have a downstream impact on future treatment options. Currently, AIT is not available for Bermuda grass in Canada. However, there are several AIT options for Timothy grass and other perennial grasses. As a result, it is unclear how these treatments may affect Bermuda grass–induced AR.

Bermuda grass–sensitized participants from Kingston, Ontario, experienced clinical AR symptoms when exposed to Bermuda grass pollen extract during NAC. The AR nasal symptoms observed were significantly different for 3 hours after NAC when compared with controls. Local eosinophilia in nasal lavage fluids was observed in allergic participants, but not in healthy controls, as seen previously with ragweed allergen challenge.³⁸ In addition, Bermuda grass sIgE levels increased between the screening and NAC visits for patients with Bermuda grass allergy. Overall, the AR-CIC NAC model is an effective and well-controlled technique to study Bermuda grass–induced AR.

Acknowledgments

The authors thank Terry Walker, Hannah Botting, Mark Tenn, Mallory Gallant, Leah Slater, Erika Wall, Alessandra Castañón, Brittany Boyd, and Janice Menzies for their assistance with this study; Wilma M. Hopman for her consultation on statistical analyses; and Babak Aberumand for his help in investigating Bermuda and Timothy grass dual sensitization in Kingston, Ontario.

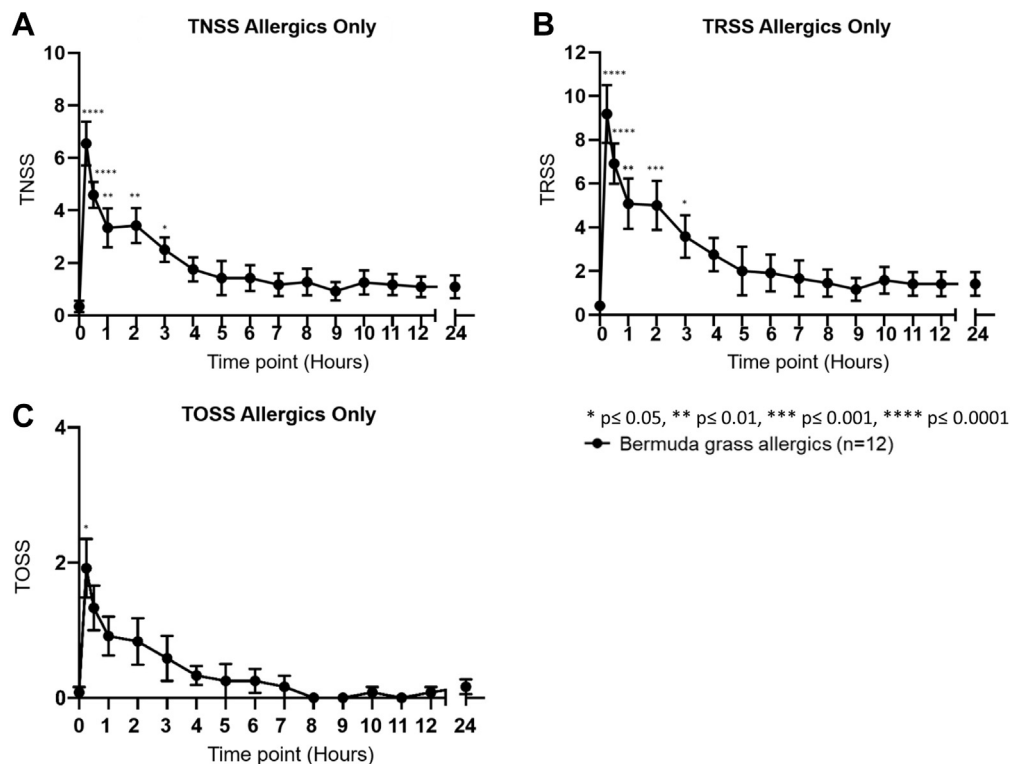
Supplementary Data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.anai.2020.03.003>.

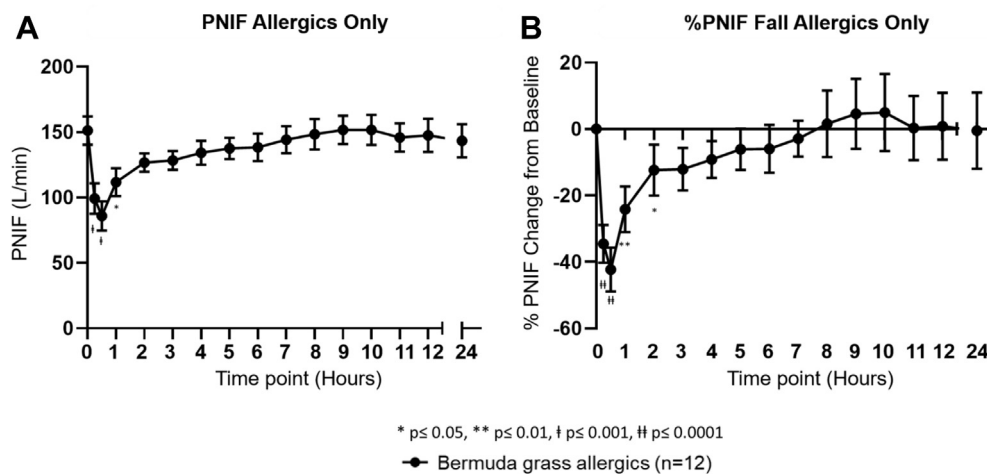
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Appendix



eFigure 1. Progression of allergic rhinitis (AR) symptoms in Bermuda grass allergic participants during the nasal allergen challenge (NAC). The mean Total Nasal Symptom Score (TNSS) (A), Total Rhinoconjunctivitis Symptom Score (TRSS) (B), and Total Ocular Symptom Score (TOSS) (C) were measured for the allergic group to examine the change from baseline for symptom scores during the NAC visit. Compared with baseline, TNSS was significantly increased for participants with allergy at 15 minutes ($P < .0001$), 30 minutes ($P < .0001$), 1 hour ($P = .0042$), 2 hours ($P = .0013$), and 3 hours ($P = .0173$) after challenge (Kruskal-Wallis test with Dunn's test). Likewise, TRSS was also significantly increased for participants with Bermuda grass allergy at 15 minutes ($P < .0001$), 30 minutes ($P < .0001$), 1 hour ($P = .0022$), 2 hours ($P = .0009$), and 3 hours ($P = .0209$) after NAC (Kruskal-Wallis test with Dunn's test). TOSS was also significantly elevated at 15 minutes after allergen challenge ($P = .0168$, Friedman test with Dunn's test).



eFigure 2. Progression of the peak nasal inspiratory flow (PNIF) in Bermuda grass allergic participants during the nasal allergen challenge (NAC) visit. The kinetic profile of nasal airflow was measured during the NAC visit using PNIF. Allergic participants had significantly decreased PNIF at 15 minutes ($P = .0007$), 30 minutes ($P = .0005$), and 1 hour ($P = .0187$) after NAC compared with baseline (one-way analysis of variance [ANOVA] with Dunnett's test) (A). Likewise, the percent PNIF fall was significantly greater at 15 minutes ($P < .0001$), 30 minutes ($P < .0001$), 1 hour ($P = .0011$), and 2 hours ($P = .0464$) after NAC (Friedman test with Dunn's test) (B).

eTable 1

Medications Not Permitted Before and During the BerCAC Study

Restricted medications	Washout period before screening visit
β -blockers	14 d
Tricyclic antidepressants	14 d
Monoamine oxidase inhibitors	14 d
Omalizumab, mepolizumab, reslizumab, duplimab, or benralizumab	6 mo

Abbreviation: BerCAC, Bermuda Grass Cumulative Allergen Challenge.

eTable 2

Restricted Medications and Washout Periods Before and During BerCAC Study Visits

Medication	Washout period ^a
Systemic (intramuscular and/or intravenous and/or oral) corticosteroids (topical hydrocortisone $\leq 1\%$ covering $\leq 10\%$ of body surface without occlusion is allowed)	30 d
Intranasal corticosteroids	14 d
Leukotriene receptor antagonists	14 d
Inhaled, intranasal or ocular cromolyn	10 d
Antiallergic ophthalmic treatments, that is, Patanol or Zaditen	3 d
H ₁ -receptor antagonists (oral and/or topical) and/or H ₂ -receptor antagonists	5 d
Nasal decongestants	24 h

Abbreviation: BerCAC, Bermuda Grass Cumulative Allergen Challenge.

^aWashout periods before screening visit or NAC visits.