

Allergen bronchoprovocation of patients with mild allergic asthma after ozone exposure

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Background: Clinical and epidemiologic studies suggest that ambient ozone exposure may increase the response of patients with asthma to inhaled allergen.

Objectives: The study was designed to evaluate whether a resting 1-hour exposure to 0.12 ppm ozone increases the sensitivity of patients with atopic asthma to inhaled allergen.

Methods: Outside of their allergen season, 15 patients with mild atopic asthma (5 women and 10 men) were exposed, on separate occasions, for 1 hour at rest to clean air and 0.12 ppm ozone. Exposures were separated by a minimum of 4 weeks in a counterbalanced, double-blind design. After exposure, subjects underwent inhalation challenge with doubling doses of aerosolized allergen (0.05 to a maximum of 1600 protein nitrogen units/ml) until we elicited a 20% FEV₁ decrement (PC₂₀).

Results: Baseline symptoms, spirometry, and histamine bronchoreactivity were similar for the two exposures. Neither spirometry results nor symptoms were significantly changed after either exposure. The mean difference in response to allergen challenge on the air and ozone days, for the 12 subjects who attained a PC₂₀, was not significant ($p = 0.124$). Three subjects required the same allergen dose to reach PC₂₀ for both exposures, five required less allergen after ozone exposure, and four required more. There was no order effect for the acute response to allergen challenges ($p = 0.325$). However, 20 hours after allergen challenge, histamine bronchoreactivity was increased ($p < 0.05$) to a similar degree for both air and ozone.

Conclusions: A resting exposure for 1 hour to 0.12 ppm ozone did not potentiate an immediate bronchoconstrictive response to grass allergen in this group of patients with mild atopic asthma. (*J Allergy Clin Immunol* 1996;98:563-72.)

Key words: Asthma, allergen, bronchial provocation, allergen challenge, ozone

Between 1970 and 1985, asthma prevalence in the United States increased in all age groups, with an overall increase of 22% from 30.2 to 36.8 per

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Abbreviations used

FVC:	Forced vital capacity
LAR:	Late asthmatic response
MDI:	Metered-dose inhaler
NSBR:	Nonspecific bronchial reactivity
PNU:	Protein nitrogen units
UNC:	University of North Carolina

1000.¹ Although the reasons for this change remain unclear, air pollution is considered a possible factor in asthma pathogenesis. Of the commonly monitored ambient air pollutants, ozone is of particular concern. Over a third of the U.S. population live in areas that are not in compliance with the current national ambient air ozone standard of 0.12 ppm for 1 hour, not to be exceeded more than once per year.² Numerous epidemiologic studies have suggested that exposure to ambient levels of ozone may contribute to a variety of respiratory

TABLE I. Subject baseline characteristics

Subject No.	Age (yr)	Sex	Race/ethnic group	Height (cm)	Weight (kg)	Baseline FEV ₁ (% pred)*	
						Air	Ozone
001	30	M	Black/Hispanic	192	85	3861 (86)	3936 (87)
003	23	F	Black	175	69	3376 (90)	3383 (90)
004	27	F	White	160	44	2569 (84)	2597 (85)
006	20	M	White	177	77	4602 (107)	4583 (107)
007	25	F	White	171	67	3891 (113)	3887 (113)
008	34	M	White	172	89	3293 (83)	3158 (79)
010	25	M	White	183	110	3890 (85)	3757 (82)
011	27	M	White	179	70	4174 (94)	4420 (100)
013	31	M	White	170	72	3376 (88)	3185 (83)
015	19	F	Black	163	86	2371 (82)	2231 (78)
017	19	F	Latin American	157	58	2794 (98)	3030 (106)
018	24	F	White	161	49	3103 (101)	2895 (94)
Mean $\pm$ SEM	25.3 $\pm$ 1.4			171.7 $\pm$ 3.0	73.0 $\pm$ 5.3	3441.7 $\pm$ 193.3	3421.8 $\pm$ 205.9

PRN, As needed; *exer*, during exercise.

*Measured immediately before exposure.

†Interpolated concentration of histamine that caused 20% decrease in FEV₁.

‡Routinely used asthma medication.

§Number of positive responses to 16 allergens indigenous to Central North Carolina.

disorders including asthma. Several studies^{3,4} have reported an association between ambient oxidant levels and the frequency of asthma attacks. Other studies in Atlanta,⁵ New Jersey,⁶ and Southern Ontario⁷ indicated that ozone levels were significantly related to hospital admissions for treatment of asthma or for hospital emergency visits. Bates and Sizto⁷ found a significant increase in asthma-related hospital admissions in Southern Ontario, which were correlated with the previous day's ozone levels. This lag between ozone exposure and asthma exacerbation suggests an augmentation of airway sensitivity or inflammation rather than an immediate effect of ozone on lung function.

Results of controlled-exposure studies also indicate that ozone exposure may adversely affect patients with asthma. Airway inflammation, a key feature of asthma pathophysiology, is induced in normal subjects by ozone exposure.^{8,9} Additionally, ozone exposure causes increased nonspecific bronchial reactivity (NSBR) and decrements in lung function in healthy subjects.^{10,11} Kreit et al.¹² showed that exercising patients with asthma, who were exposed for 2 hours to 0.4 ppm ozone, exhibited marked decrements in lung function that were significantly greater than those in healthy subjects.

The pulmonary response to ozone is dependent on concentration, ventilation, and duration of exposure. With lower ozone concentrations, ozone-

induced effects are typically only observed when ventilation is increased by exercise and duration of exposure is extended. Thus 2-hour resting exposures to 0.30 ppm ozone do not induce group mean pulmonary function decrements or respiratory symptoms in healthy subjects.¹³ However, exposures at or below 0.12 ppm ozone^{14,15} demonstrate that normal subjects, performing sustained moderate exercise for 6.6 hours, incur progressive significant decrements in lung function and increases in NSBR.

The results of both epidemiologic and clinical studies suggest that ambient ozone exposures may cause asthma exacerbations, possibly by enhancing subsequent responses to inhaled allergen. Molfino et al.¹⁶ reported the somewhat surprising observation that a 1-hour resting exposure to 0.12 ppm ozone in patients with asthma (which had no effect on lung function or airway histamine responsiveness) caused a shift in the dose-response curve to inhaled allergen challenge (ragweed or grass), such that significantly less allergen was required to induce a 15% drop in FEV₁. Despite the small sample size ($n = 7$), these results, if substantiated, would have important implications for air quality standards. In our study we reexamined the question of whether a 1-hour resting exposure to 0.12 ppm ozone potentiates the out-of-season response to grass or ragweed allergen bronchoprovocation in patients with mild atopic asthma. Although the

Baseline NSBR PC ₂₀ (mg/ml)†		Medication‡	Skin test§
Air	Ozone		
0.30	0.37	Albuterol MDI PRN	+9
0.19	0.38	Epinephrine aerosol inhalation PRN	+9
NA	0.49	Albuterol MDI PRN 1X/wk	+4
3.13	2.83	Albuterol MDI PRN	+5
0.55	0.28	Albuterol MDI PRN exer 1-3X/mo	+5
0.57	0.29	Albuterol MDI PRN	+12
0.06	0.18	Albuterol MDI PRN 3X/wk	+7
3.25	2.16	Albuterol MDI PRN	+9
5.00	2.50	Albuterol MDI PRN Epinephrine aerosol inhalation PRN	+7
0.18	0.32	Albuterol MDI PRN 1X/wk Epinephrine aerosol inhalation PRN	+8
1.48	3.27	Albuterol MDI PRN exer 3X/wk	+2
0.35	1.72	Albuterol MDI PRN exer	+7
1.37 ± 0.50	1.30 ± 0.36	Albuterol MDI PRN	+7 ± 0.8

main focus of this study was on the early asthmatic response and the allergen PC₂₀; we also monitored the late asthmatic response (LAR).

METHODS

Subjects

We studied 15 patients with asthma (5 women and 10 men), ages 18 to 35 years. All subjects underwent routine screening procedures including medical history, physical examination, serum chemistries with a complete blood count, and completion of the Minnesota Multiphasic Personality Inventory. Epicutaneous skin prick testing was performed with 16 antigens indigenous to North Carolina and control solutions of saline and histamine. Subjects were required to have a history of physician-diagnosed asthma, increased NSBR to histamine, and skin test reactivity to grass (meadow fescue) or ragweed with a corresponding seasonal history of symptoms. The subjects withheld use of any inhaled bronchodilators for 12 hours before and during testing days. Because our goal was to study patients with mild asthma, we used the following exclusion criteria: (1) use of inhaled steroids or inhaled cromolyn within the past month; (2) use of daily theophylline, oral steroids, immunotherapy, or physician-directed emergency treatment within the past year; and (3) an unmedicated FEV₁/forced vital capacity (FVC) of 70% or less. Subject characteristics, including baseline lung function, are included in Table I.

Subjects were informed of the purpose, procedures, risks, and benefits of the study; and each signed a statement of informed consent. The protocol and subject consent form were approved by the University of North Carolina (UNC) School of Medicine Committee on the Protection of the Rights of Human Subjects, and the UNC Hospitals General Clinical Research Center.

Experimental design

Each subject participated in two exposure sessions, one exposure to 0.12 ppm ozone and the other to clean air. A counterbalanced, double-blind design was used for the exposure sequence, with a minimum of 4 weeks between exposures. The subjects were not studied during their allergy season.

Protocol

The exposure sequence was as follows.

1. Two days before exposure, subjects underwent histamine NSBR testing.
2. A 1-hour resting exposure to air or ozone was immediately followed by the subject's allergen challenge.
3. After exposure, histamine testing was done 20 hours after subjects completed their allergen challenge.

On testing days, the subject's current health and asthma activity were reassessed. Baseline lung function measurements obtained before exposures included forced expiratory spirometry and body plethysmographic determinations of airway resistance and thoracic gas volume. The subject then entered the exposure chamber, which was set for the appropriate conditions. The exposure was supervised by a technician who was not informed of the chamber conditions and did not speak with investigators conducting the lung function testing and allergen challenge. After 1 hour at rest, the subject exited the chamber and again repeated the lung function measurements. A symptom questionnaire was completed both before and after the exposure. After the lung function measurements, an allergen inhalation challenge was performed. After the allergen challenge, the subject was admitted to the General Clinical Research Center for continued overnight monitoring. Subjects were treated with albuterol sulfate metered-dose inhaler

(MDI) if their symptoms warranted treatment and, if necessary, with oral prednisone. The following morning, the subject returned to the laboratory for additional lung function testing and a histamine challenge.

Chambers

The exposures were conducted in a $4 \times 6 \times 3.2$ m stainless steel chamber, maintained at 22°C and 40% relative humidity. For all exposures, the inlet air was passed through charcoal and permanganate scrubbers and high-efficiency particulate air filters. Ozone was generated by ultraviolet irradiation of recirculated chamber air. The ozone concentration in the chamber was continuously monitored by chemiluminescent ozone analyzers (Bendix Environmental & Process Instruments, Morristown, N.J.). These analyzers were calibrated daily against an ultraviolet ozone photometer (Dasibi model 1003 AH; Dasibi Environmental Corp., Glendale, Calif.). This photometer is used as a transfer standard and is referenced directly to an Environmental Protection Agency (EPA) primary ozone standard. A detailed description of the chambers and their operation systems has been published.¹⁷

Bronchial inhalation challenge with histamine

Our methodology conforms with recommendations of the Subcommittee on Bronchial Inhalation Challenges, Assembly of Allergy and Clinical Immunology¹⁸ and the Guidelines for Standardization of Bronchial Challenges with Bronchoconstricting Agents, from the European Society for Clinical Respiratory Physiology (SECRP) Working Group.¹⁹ Histamine aerosols were generated from solutions of the drug dissolved in normal saline solution by using a DeVilbiss jet nebulizer (no. 646; DeVilbiss Health Care, Inc., Somerset, Pa.). The nebulizer was operated at a pressure of 12 psi and contained 3 ml of solution. The nebulizer was attached via a T-tube, one port of which was connected to a filter and pneumotachograph (no. 3700; Hans Rudolph, Inc., Kansas City, Mo.). Histamine aerosol was generated throughout the first second of each inhalation while the subject inhaled five slow breaths (minimum volume, 1 L). Expired air and excess aerosol were exhausted through the filter. Two minutes after the start of aerosol inhalation, subjects performed two forced expiratory maneuvers. This sequence of aerosol inhalation, followed by forced expiratory spirometry, was repeated with progressively increasing concentrations of histamine at 5-minute intervals. Histamine concentrations were 0.00 (saline control), 0.075, 0.156, 0.312, 0.625, 1.25, 2.5, 5.0, and 10.0 mg/ml. The testing was terminated when the subject's FEV₁ decreased by at least 20% from the saline baseline value. Increased reactivity was defined as a 20% decrease in FEV₁ elicited by a concentration of histamine of 5.0 mg/ml or less. All subjects were monitored with pulse oximetry and electrocardiographic telemetry.

Specific allergen bronchial provocation challenge

The aerosol delivery and inhalation methods were the same as those used for the histamine bronchial challenge. A set of 16 nebulizers (DeVilbiss no. 646) was used for the allergen challenge. Nebulizers were individually calibrated by using a simulated breathing procedure. A piston respirator was connected to the nebulizers for a series of 100 breaths, and the nebulizer was weighed before and after use to determine mass output. The same nebulizer was always used with the same allergen concentration to avoid contamination. Allergen aerosols were generated from solutions of diluent (saline) containing the specific allergen (meadow fescue; Greer Laboratories, Inc., Lenoir, N.C.). After administration of the control saline aerosol, we initiated the allergen challenge with a concentration of 0.05 protein nitrogen units (PNU)/ml and proceeded with doubling doses (0.10, 0.20, 0.40, 0.80, 1.60, 3.20, 6.25, 12.5, 25, 50, 100, 200, 400, 800, and 1600 PNU/ml). Inhalations were administered at 10-minute intervals. Both allergen bronchial challenges for each subject were performed with the same nebulizers, and dilutions were prepared from the same lot of allergen.

Measurements of FEV₁ were obtained before the challenge and 8 minutes after each aerosol inhalation. The FEV₁ value obtained after the saline inhalation was regarded as the baseline FEV₁. If FEV₁ declined by less than 20% after allergen inhalation, we proceeded to the next concentration. For a few subjects whose decline approached but did not exceed 20%, the number of breaths of the next concentration was adjusted, if necessary, with the goal of achieving a FEV₁ decrement of 20% to 30%. Once a decline of at least 20% was induced, the FEV₁ measurement was repeated every 10 minutes until 30 minutes after the last dose of allergen, then every 30 minutes until 2 hours after the last dose of allergen. The maximum dose of allergen administered was the one that elicited at least a 20% FEV₁ decrement. The PC₂₀ was then calculated by linear interpolation between the FEV₁ decrements attained with the final two doses. During the allergen inhalation and postchallenge measurements, the subjects were monitored with pulse oximetry and electrocardiographic telemetry. After the allergen challenge was completed, subjects remained under physician supervision. When the subject showed stable lung function, he or she was admitted to the hospital for continued medical monitoring and hourly lung function measurements with a portable spirometer. Subjects were treated with albuterol MDI if warranted by their clinical symptoms and FEV₁ measurements.

Pulmonary function measurements and symptoms

A constant volume body plethysmograph was used to measure specific airway resistance and thoracic gas volume with a modified DuBois technique at a panting frequency of 1.5 Hz.²⁰ Three trials were performed, each

separated by 30 seconds. The two closest numerical values were used for analysis. Forced expiratory spirometric measurements were acquired, with volumes corrected to BTPS, by using a 12 L dry rolling-seal spirometer. Three trials were performed, each separated by 30 seconds. In analyzing the spirometry data, the greatest FEV₁ value and the greatest FVC value were used. Data were discarded when the maneuver was interrupted by cough. A portable hand-held spirometer (Jones Medical Instrument Co., Oak Brook, Ill.) was used for spirometric measurements in the UNC Hospital General Clinical Research Center.

A symptom questionnaire was given before exposure, immediately after exposure, and the day after. A list of 10 symptoms that are characteristic of asthma and/or ozone exposure (shortness of breath, pain on deep inspiration, breathing discomfort, chest tightness/pain, wheezing/whistling in the chest, cough, anxiety, sputum/phlegm production, irritated/sore throat, and dry mouth/throat) and five sham symptoms were presented on a computer monitor. The subject entered a number corresponding to a scale of 1 to 5, which was used for rating the severity of the symptom: 1 (none), 2 (trace discomfort), 3 (mild discomfort), 4 (moderate discomfort), and 5 (severe discomfort). The total symptom score was the sum of the severity ratings for each of the 10 symptoms.

Data management and statistical analysis

The main null hypothesis (no difference in the allergen PC₂₀ on the air and ozone exposure days) was evaluated by using a paired *t* test. With each subject serving as his or her own control (*n* = 12) in this study design, we have adequate power (an 85% probability) to detect a two-dose shift in the PC₂₀. The calculated power of detecting a one-dose shift in the PC₂₀ is only 56%. The allergen PC₂₀ values were also examined by paired *t* test to determine whether there was an order effect of the exposures. The FEV₁ decrements were evaluated for the 90 minutes after the allergen challenge, for both the air and exposure days. This response pattern was examined for a difference in the two exposure conditions by using a multivariate analysis of variance appropriate for a repeated measures design. The LAR was defined as the largest percent decrement in FEV₁ from baseline before any medication intervention, and occurring more than 4 hours after the end of the allergen challenge. A paired *t* test was used to examine the difference in the LAR to allergen challenge on the 2 exposure days.

The pulmonary function variables of airway resistance and forced expiratory spirometry were analyzed by using the measurements obtained before the histamine challenges and the pre- and postexposure measurements for the two exposures. A multivariate analysis of variance appropriate for a repeated measures design was used to determine whether there was a difference in the pulmonary function for the air and ozone exposures.

Histamine challenges were performed 2 days before and on the day after the air or ozone exposure/allergen

TABLE II. Mean pulmonary responses before and after exposure

	Air vs ozone	Before exposure	After exposure*
FVC (ml)	Air	4474 ± 293	4448 ± 269
± SEM	Ozone	4490 ± 304	4489 ± 277
FEV ₁ (ml)	Air	3442 ± 193	3465 ± 179
± SEM	Ozone	3422 ± 206	3452 ± 203
FEV ₁ /FVC (%)	Air	77.5 ± 2.3	78.8 ± 2.2
± SEM	Ozone	77.2 ± 2.5	77.5 ± 2.4
SRaw (cmH ₂ O · s)	Air	5.07 ± 0.77	4.76 ± 0.78
± SEM	Ozone	4.68 ± 0.45	4.87 ± 0.91
Histamine PC ₂₀ (mg/ml) ± SEM	Air	1.37 ± 0.50	0.85 ± 0.36
	Ozone	1.30 ± 0.36	0.66 ± 0.20

SRaw, Specific airway resistance.

*All immediately after exposure to air or ozone only, except for postexposure histamine PC₂₀ (NSBR), which was 20 hours after exposure/allergen challenge.

challenge. The PC₂₀ was calculated by using a linear interpolation between the final two doses of histamine. We tested the hypothesis that there was no difference between the pre- and postexposure PC₂₀ for histamine for the two exposure conditions.

RESULTS

Fifteen subjects were exposed to clean air and 0.12 ppm ozone, each followed by an allergen challenge with grass (meadow fescue). Three subjects who participated in the study did not develop a 20% decrement in FEV₁ with the maximum allergen dose on either exposure day, and their data were not further analyzed. After the allergen challenge, eight of 12 subjects were treated with albuterol MDI, primarily in association with their late-phase response. For three of these individuals, oral prednisone was also administered later in the evening. All exposures were separated by 4 to 5 weeks, except for three subjects with more than 5 weeks' separation.

As shown in Table I, baseline lung function on the 2 exposure days and the 2 baseline histamine PC₂₀ values were similar for each subject. The correlation coefficient for FEV₁ reproducibility was 0.98, and for the histamine PC₂₀ it was 0.82. There was no difference in group mean preexposure FEV₁ values.

Table II indicates that there was no ozone exposure effect on lung function (FEV₁ or specific airway resistance). Examination of individual data indicated that there were no individual "responders."

The results of the allergen challenge demonstrate no difference between air and ozone expo-

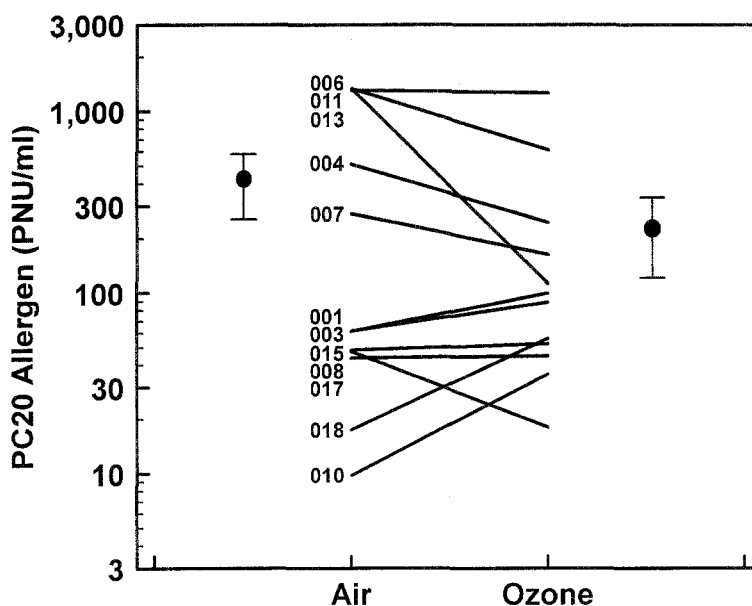


FIG. 1. Allergen PC₂₀ after air and ozone exposure. PC₂₀ allergen doses after air and ozone exposure are shown for all subjects. (Note logarithmic scale.) Each line corresponds to a different subject. The mean for air is 420.8 (SEM, 163) and for ozone, 233.2 (SEM, 105). The difference in allergen PC₂₀ between air and ozone is not significant ($p = 0.124$ [linear] and $p = 0.567$ [log-transformed]). There was no effect of the order in which the challenges were performed ($p = 0.325$.)

sure days ($p = 0.124$) in the dose of allergen required to elicit a 20% decrement in FEV₁ (Fig. 1), although there was a trend toward lower PC₂₀ after ozone exposure. The mean allergen PC₂₀, presented as mean $\pm$ SEM, was 421 ± 163 PNU/ml for the air exposure and 233 ± 105 PNU/ml for the ozone exposure. When the statistics are repeated with log-transformed data, there remains no statistically significant difference ($p = 0.567$). The challenges on the 2 exposure days never differed by more than two doubling doses except in one case ($r = 0.82$ for log PC₂₀).

Examination of the individual allergen doubling doses shows that six subjects were within one doubling dose, five subjects were within two doubling doses, and one subject was within four doubling doses. In comparing the allergen dose requirement for the two different exposures, less allergen dose was required with the air exposure for four subjects, less allergen dose was required with the ozone exposure for five subjects, and the same allergen dose was required for both exposures in three of the subjects.

One of our subjects experienced a severe asthma exacerbation in the intervening weeks between the two exposures and was unable to resume participation until he was free of symptoms for 4 weeks and until his FEV₁/FVC was greater than 70%. He

was the only subject who was not within two doubling doses for both allergen challenges; his PC₂₀ shifted by four doubling doses. When we excluded this subject from the analyses, the mean PC₂₀ was 338 ± 153 PNU/ml for air and 244 ± 114 PNU/ml for ozone compared with 421 ± 163 PNU/ml and 233 ± 105 PNU/ml. The reproducibility of the allergen challenge (log PC₂₀) also improved from $r = 0.82$ to 0.90 .

Analysis of the pattern of FEV₁ decrement from 10 to 90 minutes after allergen challenge showed no difference between the two exposures ($p = 0.152$).

In addition, there was no effect of the order of the challenges ($p = 0.325$). Eight of the subjects received air exposure first; whereas seven, including the three subjects whose allergen PC₂₀ was greater than 1600 PNU/ml, received ozone exposure first.

In our subjects the maximum LAR occurred from 4 to 8 hours after the end of the challenge and was analyzed by two approaches. Between the two exposure conditions, there was no difference in the maximum late-phase response (FEV₁ decrement) before any β_2 -agonist intervention ($p = 0.277$). With the criterion of a greater than 15% decrement in FEV₁, there was no difference in the number of subjects ($n = 4$) with a late-phase

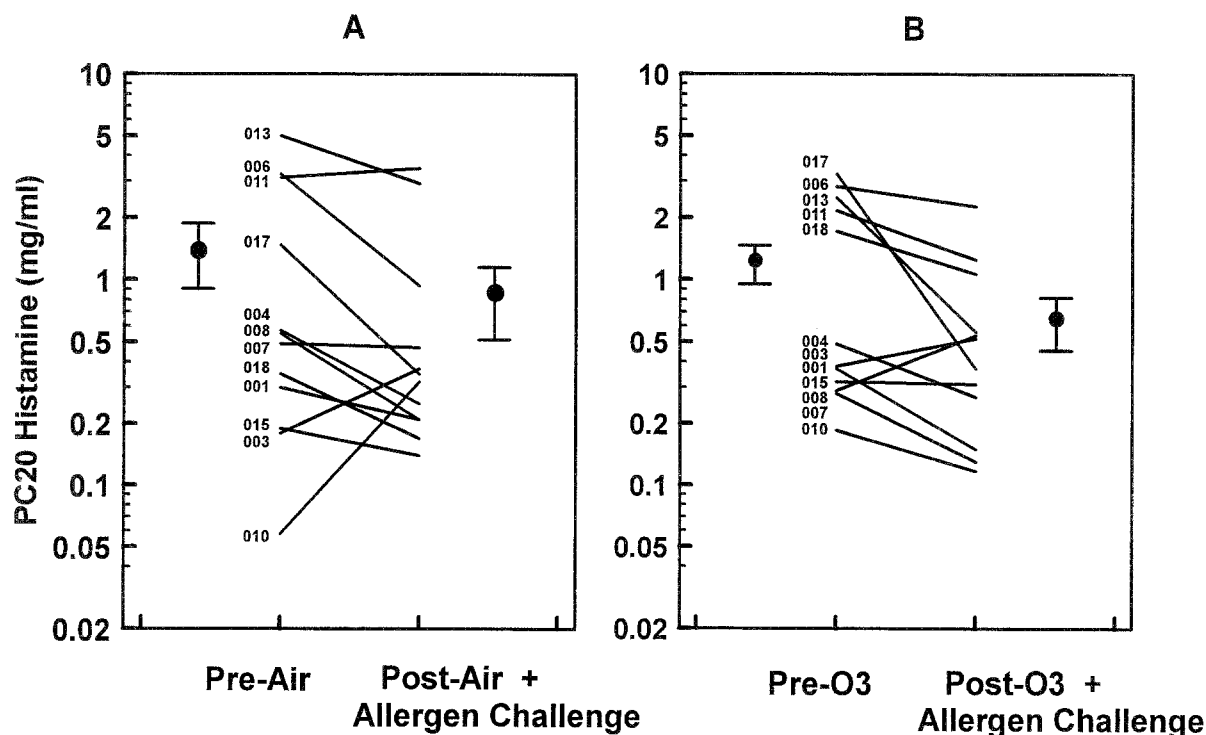


FIG. 2. Histamine PC₂₀ 48 hours before and 20 hours after exposure/allergen challenge on air and ozone days. **A**, Air exposure/allergen challenge, with each line representing a different subject. **B**, Ozone exposure/allergen challenge, with each line corresponding to a different subject. PC₂₀ histamine doses are shown on a logarithmic scale. Mean values for pre- and postchallenge values are: Pre-Air = 1.37 (SEM, 0.50) and Post-Air = 0.85 (SEM, 0.36); Pre-O₃ = 1.30 (SEM, 0.36) and Post-O₃ = 0.66 (SEM, 0.20). There was no ozone effect on PC₂₀ (Δ Air vs Δ Ozone, $p = 0.638$) and no difference in the histamine PC₂₀ baseline (pre-air vs pre-ozone, $p = 0.844$). There was a significant main effect of allergen challenge on histamine PC₂₀ ($p = 0.049$). *Pre-Air*, Before air challenge; *Post-Air*, after air challenge; *Pre-O₃*, before ozone challenge; *Post-O₃*, after ozone challenge.

response after air or ozone exposure, regardless of medication use. When the criterion of a greater than 10% decrement in FEV₁ was used, there were slightly more late-phase responses after the air exposure ($n = 7$) than after the ozone exposure ($n = 5$). However, the analysis of the late-phase responses should be considered with the caveat that seven of 12 subjects did not have the same final allergen dose (5 had a difference of 1 doubling dose, 1 had a difference of 2 doubling doses, and 1 had a difference of 4 doubling doses).

Measurements of NSBR were analyzed separately according to two main hypotheses. Postexposure NSBR, as determined by histamine bronchoprovocation, was not different ($p = 0.638$) between the air and ozone exposure (Fig. 2). In comparison with the baseline NSBR 48 hours before the allergen challenge, histamine broncho-reactivity 20 hours after the allergen challenge was significantly increased ($p < 0.05$) and to a similar

degree for both air and ozone exposure. In the latter comparison, it should be noted that eight of 12 individuals used an albuterol MDI at least once between the allergen challenge and the next day histamine challenge.

The reporting of symptoms characteristic of asthma and/or ozone exposure followed the same trend as NSBR. The total symptom scores measured before exposure were 12.2 (air) and 11.1 (ozone). Immediately after exposure, the symptom totals were 12.3 (air) and 10.8 (ozone). Twenty-four hours after the allergen challenge (just before the post-NSBR testing), symptom scores were increased for both exposures and showed a greater increase after the air exposure/challenge (16.3) than after the ozone exposure/challenge (12.8). Even with the sham symptoms included, there was still a greater increase after the air exposure/challenge (22.6) than after the ozone exposure/challenge (18.6).

DISCUSSION

The primary goal of our study was to evaluate the effect of a 1-hour resting exposure to 0.12 ppm ozone on the response of patients with mild atopic asthma to allergen challenge. Using a double-blind counterbalanced study design, we found no significant difference in the concentration of allergen required to elicit a 20% decrement in FEV₁ (PC₂₀) between the air and the 0.12 ppm ozone exposures. We also found no difference in LAR to allergen challenge between the 2 exposure days. However, an effect could have been masked by our protocol, which was not specifically designed to examine the LAR, because we did not use identical allergen doses on both days in seven of 12 subjects, and we did not restrict medical intervention to treat the associated symptoms.

As observed in other studies,²¹ NSBR increased the day after both allergen challenges. The ozone exposure did not alter the magnitude of these allergen-induced changes in NSBR. Of note, the two subjects who received prednisone after one of the two allergen challenges showed less of an increased NSBR. There was no overall difference in steroid use with regard to the ozone and air exposures. The within-subject reproducibility of the effect of allergen challenge on NSBR compares favorably with the findings of Cockcroft et al.²² despite the fact that allergen doses were not identical in the two exposures. As stated previously, it is possible that our approach masked a difference between the 2 exposure days because they were not designed to directly address the LAR and its sequelae.

Our results differ from those reported by Molfino et al.¹⁶ They reported a significant increase in the airway response to inhaled allergen of patients with mild asthma after ozone exposure. However, it is not clear that the increased allergen response was attributable to ozone rather than to repeated exposure to allergen. In the study by Molfino et al.,¹⁶ the air exposure preceded the ozone exposure in six of the seven subjects, and in four of the subjects the time interval between allergen challenges was less than 4 weeks. In support of this conjecture, the only subject who showed greater allergen responsiveness after air exposure underwent the ozone exposure first. As previously noted, we did not observe an ordering effect in the response to allergen in our study, which we attribute to the minimum 4 weeks' separation between challenges.

Although ozone exposure conditions were the same, it is possible that differences in the subject

groups account for the dissimilar results of the two studies. For the most part, the two studies are very comparable. In both studies men and women were included, testing occurred outside their allergy seasons, and the timing of the allergen challenge was immediately after the exposure. There is an age difference between the two groups; the mean age of participants in our study was 15 years less than that of the participants in the study by Molfino et al.¹⁶ However, at least with regard to the FEV₁ response, ozone-induced decrements decline with increasing age.²³ Both studies selected patients with mild asthma and similar baseline lung function (percent predicted FEV₁), but the subjects in the study by Molfino et al.¹⁶ had increased responsiveness on baseline NSBR testing (0.39 mg/ml vs 1.34 mg/ml). This may be due to methodological differences in the methacholine challenge testing.

Although there is a trend toward an ozone-induced change in allergen response, this is largely driven by one subject. Our negative findings are supported by the contemporary work of Chen et al.,²⁴ who found no significant difference in the PC₁₅ responses between allergen challenges after air and 0.2 ppm ozone exposures for 1 hour during exercise. Because a 1-hour resting exposure to 0.12 ppm ozone has no measurable effects on lung function or NSBR in normal subjects, our findings were not unexpected. However, there is substantial basis to expect that with more vigorous exposure conditions, an ozone-induced enhancement of the response to allergen inhalation may be observed. We would anticipate that ozone exposure conditions sufficient to increase NSBR may be necessary to augment the early bronchoconstrictive response to allergen, which is partially mediated by mast cell degranulation and histamine release. Jörres et al.²⁵ reported sensitivity to inhaled allergen increased by at least one doubling dose in 18 of 23 patients with allergic asthma after 3 hours of exposure to 0.25 ppm ozone with intermittent exercise.

There are several mechanisms by which ozone exposure could augment the response of patients with asthma to inhaled allergen. For example, recent work from our laboratory has shown that exposure to 0.4 ppm ozone for 2 hours augments allergen-induced eosinophil influx and activation in the nasal airways of patients with perennial house dust mite allergy and asthma.²⁶ Ozone alone, without allergen, also yields an eosinophilia. Illustrating this point is a study of patients with asymptomatic allergic rhinitis exposed to 0.5 ppm ozone for 4 hours, reporting a mixed inflammatory

cell influx in nasal lavage fluid.²⁷ They had a predominance of neutrophils and increases of eosinophils and mononuclear cells on the ozone exposure day. Additionally, ozone increased eosinophils and eosinophil cationic protein, albeit at lesser levels than the combination of ozone and allergen as described previously. This inflammatory cell influx and activation after ozone exposure alone mimics, to a certain extent, the effects of allergen applied to the nasal mucosa of allergic subjects. If ozone exerts a similar effect in the lower airways, the chronic increase of airway eosinophils may cause worsening of asthma.

It is also likely that ozone exposure increases oxidative stress in the airways. Patients with asthma have been shown to have increased oxidant stress in the airways, as demonstrated by increased exhaled hydrogen peroxide.²⁸ Furthermore, the release of neuropeptides from nonadrenergic, noncholinergic neurons may contribute to bronchospasm and mucosal edema of patients with asthma. Allergen challenge of the airways induces release of neuropeptides; and oxidant stress inactivates neutral endopeptidase, an airway epithelial surface enzyme, which degrades neuropeptides. Thus ozone exposure may alter homeostatic mechanisms in the airways.

As shown both by accelerated pulmonary clearance of technetium Tc 99m-labeled diethylenetriaminepentaacetic acid²⁹ and increased protein in bronchoalveolar lavage fluid,⁸ ozone exposure increases respiratory epithelial permeability in healthy subjects. Epithelial cells act as a barrier in the airways and likely modulate movement of airborne allergen to intraepithelial mast cells and other cells, which mediate the allergen responses. Conceivably, ozone could alter responsiveness of patients with asthma to inhaled allergen by increasing transepithelial permeability. Such an effect would increase the delivery of allergen to mast cells, augmenting both immediate changes in airway responsiveness to allergen and later infiltration with inflammatory cells such as neutrophils and eosinophils.

Despite recent interest in the ability of ozone to augment allergen-induced responses of patients with asthma, the levels of ozone required to exert such effects remain undefined. Review of both epidemiologic and controlled exposure studies suggests that ozone exposures above the current National Ambient Air Quality Standard do influence airway responses in patients with asthma. However, our data do not support the hypothesis that exposure to 0.12 ppm ozone for 1 hour,

without exercise, alters bronchial responses to inhaled allergen in patients with mild grass allergy and asthma studied out of season. Further studies are needed to define the parameters of ozone exposure likely to adversely affect this potentially sensitive population.

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