

Increased specific airway reactivity of persons with mild allergic asthma after 7.6 hours of exposure to 0.16 ppm ozone

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Background: Exposure to ozone causes decrements in lung function, increased airway reactivity to nonspecific bronchoconstrictors, and lung inflammation. Epidemiology studies show an association between ambient oxidant levels and increased asthma attacks and hospital admissions.

Objective: The purpose of our study was to evaluate the response of persons with mild asthma to inhaled allergen after ozone exposure conditions similar to those observed in urban areas of the United States.

Methods: Using a double-blind, counter-balanced design, we exposed 9 (5 women and 4 men) subjects with mild atopic asthma (house dust mite sensitive) to clean air and to 0.16 ppm ozone for 7.6 hours; exposures were separated by a minimum of 4 weeks. During exposure, subjects performed light exercise (ventilation = 24 L/min) for 50 minutes of each hour, and pulmonary function was evaluated before and after exposures.

The morning after exposure, subjects underwent bronchial challenge with inhaled house dust mite allergen (*Der-matophagoides farinae*). Using a series of doubling allergen concentrations, subjects inhaled 5 breaths of nebulized allergen (0.06 to 500 AU/mL) at 10-minute intervals until a minimum of a 20% decrement in FEV₁ was elicited.

Results: Compared with the change in FEV₁ during air exposure, there was a mean 9.1% ± 2.5% (SEM) decrement in FEV₁ observed because of ozone ($P < .01$). Seven of the 9 subjects required less allergen after ozone exposure than after air exposure; there was a 0.58 mean dose shift in the doubling concentration of allergen attributable to the ozone exposure ($P = .03$).

Conclusion: These findings indicate that exposure of subjects with mild atopic asthma to ozone at levels sufficient to cause

modest decrements in lung function also increases the reactivity to allergen. To the extent that this effect occurs in response to ambient exposures, ozone may be contributing to the aggravation of asthma. (*J Allergy Clin Immunol* 1999;1198-204.)

Key words: Asthma, allergen, bronchial provocation, ozone

Asthma affects approximately 18 million people in the United States and is among the few common childhood illnesses for which morbidity, mortality, and prevalence are all rising; it is estimated that 8% to 12% of inner-city children have asthma.¹ Air pollution is considered a likely factor in the exacerbation of asthma, and the effect of atmospheric pollutants on the health and respiratory function of asthmatic subjects has been the focus of numerous epidemiologic and clinical studies. Ozone, the major component of photochemical pollution, is of particular concern both because of the breadth of the population exposed and the known effects of ozone toxicology on the respiratory tract. In 1995, over 70 million people in the United States resided in areas that were noncompliant with the national ambient air quality standard of 0.12 ppm ozone for 1 hour.² In 1997, the Environmental Protection Agency tightened the standard for ozone, and it is now based on an 8-hour average of 0.08 ppm ozone.

In clinical studies of normal subjects, exposure to ozone induces decrements in lung function with increases in nonspecific bronchial hyperresponsiveness (BHR) and airway inflammation, even at levels as low as 0.08 ppm.³⁻⁶ Contrary to expectations, initial studies of persons with asthma at low ozone concentrations generally failed to demonstrate bronchoconstriction or substantially larger acute ozone-induced decrements in spirometric function than found in healthy subjects.⁷ However, in a study that used a high inhaled dose of ozone, Kreit et al⁸ showed increased airways resistance and larger decrements in FEV₁ and FEV₁/forced vital capacity (FVC) of subjects with asthma than that found in normal subjects. Similarly, in a recently completed study of subjects with moderate asthma performing light exercise while undergoing a 7.6-hour exposure to 0.16 ppm ozone, we observed larger FEV₁ decrements and more bronchoconstriction than in a cohort of similarly exposed normal subjects.⁹

Numerous epidemiologic studies have shown an association between exposure to ambient ozone and exacerbations of a variety of respiratory disorders, including

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Abbreviations used

BHR:	Bronchial hyperresponsiveness
BTPS:	Body temperature pressure standard
FVC:	Forced vital capacity
GCRC:	General Clinical Research Center
UNC:	University of North Carolina

asthma. Several studies^{10,11} have reported an association between ambient oxidant levels and the frequency of asthma attacks. Other studies in Atlanta,¹² New Jersey,¹³ and Southern Ontario¹⁴ indicated that ozone levels were significantly related to hospital admissions or hospital emergency visits for asthma exacerbations. Bates and Sizto¹⁴ 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 airways sensitivity or inflammation rather than an immediate effect of ozone on lung function as responsible for the increased hospital admissions. These observations provide a substantial basis to suspect that ozone exposure not only causes respiratory inflammation and an increase in BHR but may also enhance the response of persons with asthma to inhalation of specific allergen. Exposure studies examining the effect of low levels of ozone (<0.2 ppm for ≤ 2 hours) have yielded conflicting results as to the ability of this gas to enhance response to inhaled allergen.¹⁵⁻¹⁷ However, exposure to a higher effective ozone level clearly demonstrated that ozone enhances responses to allergen.¹⁸

This is the third in a series of studies examining the effect of ozone on persons with asthma that uses an ozone exposure regimen shown in the initial study to cause an FEV₁ decrement of 18% in a cohort of subjects with moderate asthma.⁹ This regimen incorporates prolonged duration, light exercise, and level of ozone exposure (0.16 ppm) to simulate conditions encountered in the ambient atmosphere. The second study of this series evaluated the effect of this exposure regimen on both lung function and airway inflammation of subjects with allergic asthma 18 hours after exposure.¹⁹ In this third study we examine the effect of ozone on immediate and late-phase bronchial responsiveness of subjects with mild allergic asthma to inhaled *Dermatophagoides farinae* allergen given 18 hours after exposure.

METHODS

Subjects

The subjects were 9 individuals with mild atopic asthma (5 women and 4 men) aged 20 to 35 years old. Subjects underwent a complete medical screening, including a medical history, physical examination, chemistry panel, and complete blood count with differential. Each subject also underwent epicutaneous prick tests with 0.05 mL (1:20 wt/vol) each of 18 antigen extracts, as well as histamine and normal saline controls; the antigen panel included *D farinae* and other allergens indigenous to the Southeastern United

States. Positive skin test reactivity was defined as a skin prick test-induced wheal response at least 3 times the size of that produced by the saline control. All subjects showed skin test reactivity to multiple allergens, as well as meeting the qualification criterion of showing specific allergy to *D farinae*. Because our intent was to study subjects 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, (3) use of oral steroids within the past 6 months, (4) immunotherapy, (5) physician-directed emergency treatment within the past year, and (6) an unmedicated FEV₁/FVC of less than 65%. 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 General Clinical Research Center (GCRC). For female subjects, evidence for the absence of pregnancy was initially determined by menstrual history during the screening procedures and subsequently confirmed with a urine pregnancy test before testing. Fifteen subjects were accepted into the study; 5 did not proceed past the training and 1 did not return for the second exposure. The characteristics of our 9 participants, including baseline lung function, are shown in Table I. Subjects showed minimal baseline airflow obstruction, and medication use was limited to occasional inhaled β -agonist bronchodilator. Study participants were studied when baseline lung function was stable, and they reported minimal symptoms of active allergy or asthma.

Study protocol

Each subject participated in 2 exposure sessions, one session to 0.16 ppm ozone and the other exposure to clean air. The subjects withheld use of any inhaled bronchodilators for 12 hours before testing days. The exposure protocol used was the same as that outlined by Horstman et al.⁹ The order of exposures was counterbalanced for the group of subjects, and the 2 exposures were separated by a minimum of 1 month; neither investigators nor subjects were apprised of the exposure condition. One to 3 days before an exposure, subjects underwent testing of histamine BHR. On the exposure day, subjects performed seven 50-minute exercise periods on the treadmill and cycle ergometer; during each hour, subjects exercised for 25 minutes on a treadmill followed by 25 minutes on a cycle ergometer. They had an additional 35-minute lunch break (total rest time 45 minutes) after the third exercise period for a total exposure duration of 7.6 hours. During each hour, ventilation, tidal volume, and respiratory frequency were measured during 2 minutes of each exercise task (treadmill and cycle ergometer), and exercise intensity was adjusted to achieve a ventilation rate of 25 L/min (13-15 L[$\text{min} \cdot \text{m}^2$] body surface area). While in the chamber, subjects were continually monitored by means of closed circuit television, direct observation, and electrocardiographic telemetry. Subjects performed spirometry before exposures and after each exercise period. Subjects were asked to refrain, but were not prohibited from using, inhaled bronchodilators during exposures.

The morning after the exposure day, subjects returned to the laboratory for the allergen bronchial inhalation challenge and were subsequently admitted to the GCRC of the UNC hospitals. The following morning, subjects returned to the laboratory for additional lung function testing and a histamine challenge. The next day, postallergen histamine challenge was performed only if and when each subject's FEV₁ was within 10% of his or her pre-exposure baseline.

Chambers

The exposures were conducted in a 4 $\times$ 6 $\times$ 3.2-m stainless steel chamber, which was maintained at 22°C and 40% relative humidity

TABLE I. Subject characteristics

Subject	Sex	Age (y)	FEV ₁ /FVC (%)	BHR*	House dust mite†	β-Agonist‡
6	F	23	76	0.54	8 × 10	wk
7	F	22	85	4.93	10 × 10	Rare
8	M	20	78	0.37	10 × 12	wk
9	F	26	89	1.99	7 × 25	None
10	F	35	73	0.56	10 × 15	wk
12	M	21	79	2	10 × 12	Rare
13	F	27	83	1.6	11 × 15	mo
14	M	26	74	0.82	6 × 10	None
15	M	21	80	0.58	8 × 10	wk

*Histamine PC₂₀ mg/mL.†Skin test response to *D Farinae* in millimeters.

‡Use of inhaled bronchodilator: rare <1/mo; 1/mo ≤ mo < 1/wk; wk ≥ 1/wk.

throughout all sessions. During exposures, inlet air was passed through charcoal and permanganate scrubbers and high-efficiency particle air filters. Ozone was generated by UV irradiation of recirculated chamber air. The ozone concentration in the chamber was continuously monitored by Bendix chemiluminescent ozone analyzers to insure that pollutant gas concentration did not show a greater than ±5% variance from the predetermined level. These analyzers were calibrated daily against a UV ozone photometer (Dasibi model 1003 AH). This Dasibi photometer is used as a transfer standard and is referenced directly to an Environmental Protection Agency primary ozone standard. A detailed description of the chambers and their operation systems has been published.²⁰

Bronchial inhalation challenge with histamine

Our methodology conforms with recommendations of the Subcommittee on Bronchial Inhalation Challenges of the American Thoracic Society²¹ and the Guidelines for Standardization of Bronchial Challenges with Bronchoconstricting Agents.²² Histamine aerosols were generated from solutions of the drug dissolved in normal saline by using a DeVilbiss jet nebulizer (#646) controlled by a Rosenthal dosimeter. The nebulizer was operated at a pressure of 20 psi and contained 3 mL of solution. Dosimeter settings were for 0.3-second inspiratory delay and a nebulization time of 1.0 second. Subjects inhaled 5 slow, near-vital-capacity breaths; expired breath and excess aerosol were exhausted through a filter connected to the exhaust port of the dosimeter tube. Three minutes after the start of aerosol inhalation, subjects performed 2 forced expiratory maneuvers. This sequence of aerosol inhalation followed by forced expiratory spirometry was repeated by using 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 responsiveness was defined as a 20% decrease in FEV₁ elicited by a concentration of histamine of 5.0 mg/mL or less. Subjects were monitored with pulse oximetry and electrocardiographic telemetry during the histamine bronchial challenge.

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 #646) were 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 to determine mass output. The same nebulizer was always used with the same allergen concentration to avoid cross-contamination. Allergen aerosols were generated from solutions of diluent (saline) containing the *D farinae* allergen (Greer Laboratories) measured in allergen units. After the control saline aerosol, we initiated the allergen challenge with 0.06 AU/mL and proceeded with doubling doses (0.12, 0.25, 0.50, 1.0, 2.0, 4.0, 8.0, 16, 32, 64, 125, 250, 500, 1000, and 2000 AU/mL). Inhalations were administered at 10-minute intervals. Both allergen bronchial challenges for each subject were performed by using the same nebulizers, and dilutions were prepared from the same lot of allergen.

Two measurements of FEV₁ were obtained before the challenge and 8 minutes after each aerosol inhalation. The largest FEV₁ obtained after the saline inhalation was regarded as the baseline FEV₁. When FEV₁ declined by less than 10% after allergen inhalation, we proceeded to the next concentration. If the FEV₁ decline was between 10% and 20%, spirometry was repeated until the maximum decline was achieved. If the decline remained less than 20%, the number of breaths of the next concentration was adjusted if necessary, with the goal of achieving an FEV₁ decrement of 20% to 30%. Once a minimum decline of 20% was induced, the FEV₁ measurement was repeated every 10 minutes until 30 minutes and then every 30 minutes until 2 hours after the last dose of allergen. During the allergen challenge and immediate follow-up period, the subjects were under physician supervision and were monitored with pulse oximetry and electrocardiographic telemetry. When subjects showed stable lung function, they were admitted to the UNC Hospital GCRC for continued medical monitoring and hourly lung function measurements with a portable spirometer. After allergen challenge, subjects were routinely treated with inhaled albuterol when they experienced a greater than 20% decrement in FEV₁. Those who did not respond to inhaled albuterol or experienced repeated declines in spirometric function were administered 60 mg of oral prednisone.

Measurement methods

Before and during exposures, forced expiratory spirometric measurements were acquired with a pneumotachograph (Hans Rudolph #3800) connected to a differential pressure transducer (±2 cm H₂O) interfaced to a computer (Medical Graphics System #1085); volumes were corrected to the body temperature pressure standard (BTPS). For the allergen and histamine bronchial challenges, FVC maneuvers were performed on a 12-L dry rolling seal spirometer (CPI model 220), with volumes corrected to the BTPS. While subjects were in the GCRC, a portable hand-held spirometer (Jones Medical Instrument Company) was used for spirometric measurements, with volumes corrected to the BTPS. For measurements

TABLE II. Changes in FEV₁ during clean air and 0.16 ppm ozone exposure

Subject	Clean air			0.16 ppm ozone			XΔ
	Before, L	Δ, L	(%)	Before, L	Δ, L	(%)	(%)
6	3.35	0.08	(2.4)	3.22	-0.20	(-6.2)	-8.6
7	3.39	0.04	(1.2)	3.54	-0.16	(-4.5)	-5.7
8	4.18	0.39	(9.3)	4.11	0.24	(5.8)	-3.5
9	2.49	0.03	(1.2)	2.44	-0.02	(-0.8)	-2.0
10	3.23	0.29	(9.0)	3.28	-0.23	(-7.0)	-16.0
12	3.9	-0.07	(-1.7)	3.59	-1.01	(-28.1)	-26.3
13	3.39	0.16	(4.7)	3.28	-0.12	(-3.7)	-8.4
14	4.15	-0.11	(-2.7)	4.05	-0.32	(-7.9)	-5.3
15	4.3	-0.22	(-5.1)	3.83	-0.42	(-11.0)	-5.8
Mean	3.6	0.07	(2.0)	3.48	-0.25	(-7.0)	-9.1

acquired concurrently, the mean FEV₁ values obtained from the 3 spirometers were within 5% of each other.

Data management and statistical analysis

The main hypotheses tested were that ozone exposure would induce a significant decrement in FEV₁ and that ozone would induce increased specific airway reactivity to inhaled allergen 18 hours after exposure. The relationship of ozone exposure to other lung function measurements, symptoms, BHR to histamine, and late-phase response to allergen were considered exploratory hypotheses. The largest FEV₁ of each set of measurements obtained during exposures and bronchial challenges was used for data analysis. The concentrations of allergen and histamine were log transformed, and the PC₂₀ was calculated by linear interpolation of the line determined by the final 2 allergen-histamine doses versus change in FEV₁. Data are presented as both dose number and concentration. Allergen responsiveness data were evaluated for both exposure and ordering effects. The occurrence of a late-phase response was defined as a greater than 20% decrement in FEV₁ more than 4 hours after the allergen challenge. The response to histamine was evaluated 1 to 3 days before the exposure and again the morning after the allergen challenge. We compared baseline histamine BHR, as well as testing the hypothesis of no change from the pre- to post-BHR values both across and between the 2 exposure conditions.

Each data set was tested for normality, and all were found to be normally distributed. For the paired measurements observed from each individual, statistical analysis for a given endpoint was made by using 2-tailed parametric tests (*t* tests). Significance was set at a *P* value of less than .05, although *P* values of greater than .05 but .10 or less are noted parenthetically. Correlation between lung function and allergen bronchial responsiveness changes observed during ozone exposure was evaluated with linear regression analysis. All statistical analysis was performed by using GraphPad Prism software (Version 2.0, GraphPad).

RESULTS

Subject characteristics are shown in Table I. Nine subjects participated in the study, and they were all sensitive to the allergen challenge; 4 subjects were initially exposed to ozone, and 5 underwent the air exposure first. None of the subjects used medications during exposures. Baseline lung function was stable from the pre-exposure testing of histamine BHR to the exposure days. As shown in Table II, baseline lung function was the same for each subject on the 2 exposure days. Compared with the air-

exposure day, subjects incurred a mean decrement in FEV₁ of 9.1% because of ozone exposure (*P* < .01). As observed in studies of normal subjects, there was marked individual variability, with responses ranging from 2% to 26%.

The allergen PC₂₀ values observed after the exposures are shown in Fig 1 and Table III. Seven of the 9 subjects required less allergen to elicit a 20% decrement in FEV₁ after the exposure to ozone than after air exposure. Overall, there was a mean dose shift of 0.58 attributable to the ozone exposure in the doubling concentration of allergen (*P* = .03). There was no allergen-challenge ordering effect (*P* = .65). After the allergen challenges, 4 subjects were treated with a β₂-agonist inhaler, 3 on both days and 1 on a single day. One subject also received 60 mg of prednisone after both challenges. In our subjects the maximum late-phase response occurred between 4 and 12 hours after the end of the challenge. Four subjects incurred late-phase responses. Three subjects showed late-phase responses after both allergen challenges, and 1 subject met late-phase criteria only after the air exposure.

Complete histamine BHR data were acquired from 8 subjects and are shown in Fig 2. Pre-exposure determinations of histamine BHR were not significantly different for the 2 exposure conditions. In comparison with the pre-exposure BHR, histamine responsiveness was increased after the air-allergen exposure (*P* < .05) but not after the ozone-allergen exposure (*P* = .10). It should be noted that subjects received less allergen after ozone than air exposure.

DISCUSSION

The results of this study demonstrate that exposure to 0.16 ppm ozone for 7.6 hours with light exercise (ventilation = 25 L/min) causes increased airway responsiveness to inhaled mite allergen in a cohort of 9 persons with mild atopic asthma. Our findings are consistent with the work of Jorres et al¹⁸ and suggest that ozone exposure conditions capable of inducing lung function decrements also increase airway responsiveness to specific allergen in subjects with mild atopic asthma. Whereas we evaluated allergen response 18 hours after exposure, in the study by Jorres et al, subjects underwent allergen chal-

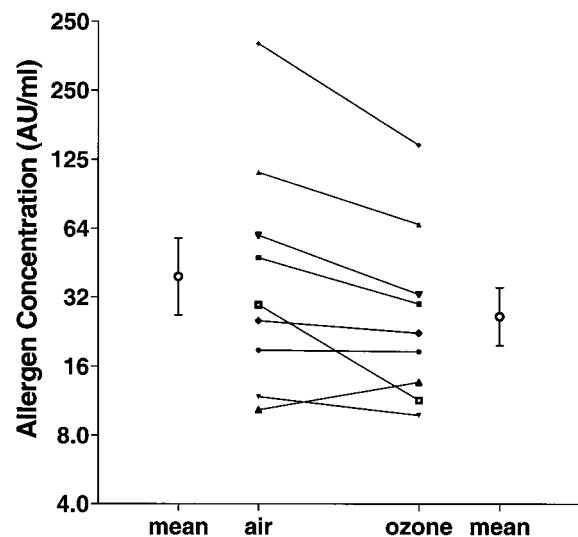


FIG 1. PC₂₀ allergen doses after the air and ozone exposure are shown for all subjects (note the logarithmic scale). Each symbol represents a different subject. The mean for air and for ozone are shown by an open circle with error bars depicting SEM. Seven subjects required less allergen after the exposure to ozone with a mean dose shift of 0.56 in the doubling concentration of allergen ($P = .03$). There was no allergen-challenge ordering effect ($P = .65$).

lenges 3 hours after the exposure. Thus the ozone-induced increased allergen responsiveness appears to persist for a minimum of 15 hours.

The mechanism by which ozone alters immediate-phase responsiveness to inhaled allergen in subjects with asthma is uncertain. It is possible that the effect of ozone on such allergen responses is directly due to the ozone-induced increase in BHR shown with both cholinergic agonists and histamine.^{3,8} Such an explanation is feasible because the immediate-phase response to allergen is mediated primarily by histamine released by mast cells. Yet the work by Jorres et al¹⁸ showed no correlation between ozone-induced changes in BHR and changes in allergen responsiveness. In their study they evaluated airway responsiveness to methacholine 1 hour before and 1 hour after exposures and allergen responsiveness 3 hours after exposure. Their findings argue against an increased BHR to the histamine released by mast cells during the immediate-phase response to allergen as the mechanism for the observed increase in allergen response.

A second hypothesis is that ozone induces changes in the airway that enhance mast cell degranulation responses to allergen. Kehrl et al²⁴ have shown that a robust exposure to ozone causes increased respiratory epithelial permeability in normal subjects. Such a change could increase the amount of allergen that can interact with mucosal mast cells sensitized to that allergen with specific IgE. Additionally, increases in fibronectin levels in bronchoalveolar lavage fluid have been reported in normal subjects exposed to ozone.²⁵ Fibronectin has been shown to enhance IgE-dependent degranulation responses of a rat mast cell line in vitro.²⁶ The ozone-induced

TABLE III. Response to house dust mites after exposure to clean air and ozone

Subject	Clean air			0.16 ppm ozone		
	PC ₂₀ (AU/mL)	EAR	LAR	PC ₂₀ (AU/mL)	EAR	LAR
6	48	29	26*	30	21	22*
7	110	22	4	67	19	-2
8	12	28	35*	10	45	47*
9	399	28	4	145	46	0
10	19	43	35*	18	19	10
12	30	22	50*	11	37	20*
13	10	26	1	14	25	2
14	60	21	1	33	19	12
15	25	28	11	22	33	16
Mean	79	27	18	39	29	14
SEM	41	2.2	6.2	14	3.8	5

Early and late responses are presented as percentage decrement from baseline.

EAR, Early asthmatic response; LAR, late asthmatic response.

*Met LAR criteria of 20% FEV₁ decrement or greater.

increases in fibronectin appear to be greater 18 hours after exposure rather than 1 hour after exposure in normal subjects.²⁵ This fibronectin time course is consistent with the exposure-allergen challenge sequence used in this study. Thus ozone exposure might enhance in vivo mast cell degranulation responses.

However, other studies do not support the notion that ozone enhances mast cell responses to allergen. Studies in allergen-sensitized dogs demonstrate that ozone exposure decreases, rather than increases, immediate-phase response to allergen.²⁷ In vitro studies of ozone-exposed mast cells also suggest that this pollutant decreases IgE-mediated degranulation responses.²⁸ Likewise, preliminary data examining the effect of 0.4 ppm ozone exposure on allergen-induced nasal tryptase reveal no effect of ozone on mast cell degranulation responses.²⁹ Overall, the effect of ozone on mast cell responses to allergen remains unclear.

An alternative hypothesis to explain the effect of ozone on asthma morbidity that takes into account the lag time described above is based on the proinflammatory effect of ozone rather than the immediate effect on lung function. Ozone, at concentrations as low as 0.08 ppm, has been shown in normal subjects to induce respiratory tract inflammation characterized by influx of neutrophils.³⁰ Studies by Basha et al³¹ and Scannell et al³² suggest that subjects with asthma have increased neutrophil influx relative to normal subjects after ozone exposure. In persons who have both asthma and perennial allergic rhinitis, ozone induces nasal eosinophil influx.³³ Studies by Peden et al¹⁹ and Frampton et al³⁴ indicate that ozone exposure causes influx of eosinophils into the lower airway as well. The work by Peden et al used the same exposure protocol and subject selection criteria as in the present study. It seems plausible that inflammatory changes caused by ozone could enhance responsiveness to inhaled allergen in persons with aller-

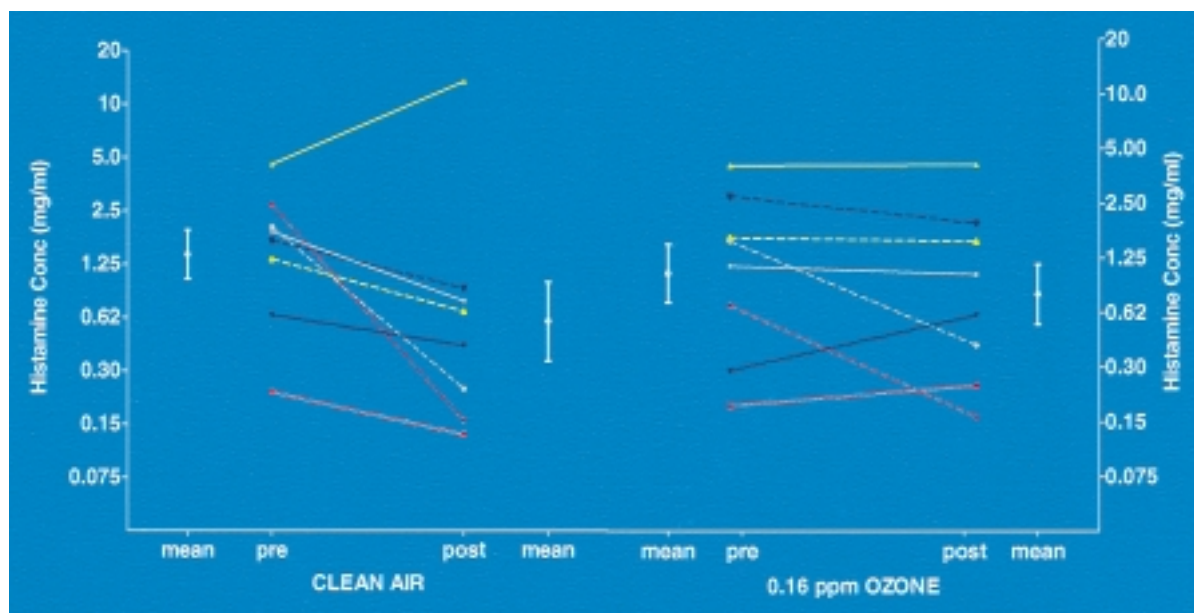


FIG 2. The *left panel* shows the histamine PC₂₀ values before air exposure and 24 hours after the allergen challenge for 8 subjects. The *right panel* similarly depicts histamine PC₂₀ values before and after the ozone-allergen sequence. Each combination of color and line (dashed versus solid lines) represents a different subject. The mean for values before and after exposure are shown by the white solid circle with error bars depicting SEM. Histamine doses are shown on a logarithmic scale.

gic asthma. Such a phenomenon would be consistent with a scenario of exposure to ozone during the late afternoon enhancing airway responses to indoor allergens encountered later that day, with any resulting change in asthma morbidity not becoming evident until the next day.

However, in our study, when compared with air exposure and with the caveat that subjects received less allergen after their ozone exposure, ozone did not exert an observable increase in either late-phase changes or enhancement of nonspecific bronchial reactivity after allergen challenge. These results are consistent with preliminary data reported by Chen et al,¹⁷ which fail to show an effect of 0.2 ppm ozone for 1 hour on either immediate-phase responses to allergen or late-phase allergen-induced inflammation in subjects with mild asthma. Even when ozone is shown to exert an effect on immediate-phase bronchial reactivity to allergen, there has yet to be reported an effect on the late-phase response, although there are reports of increased late-phase effects in the nasal airways of allergic subjects.

Although the studies cited above indicate that ozone influences airway responses to allergen in persons with asthma, it is clear that there are substantial gaps in our understanding of this phenomenon. One serious drawback to studies of the interaction of pollutants and allergen exposure in persons with asthma is that such studies predominantly use individuals with mild disease. It is persons with moderate and severe asthma, rather than those with mild disease, who are more likely to require medical attention or increase medication use after pollutant exposure. One might speculate that the airway

inflammation of those with moderate or severe disease may differ either quantitatively or qualitatively from persons with mild asthma and that these differences could increase their risk to pollutant exposure. Such differences may be profound in the airway epithelium. In vitro studies of epithelial cells have shown that many of the same changes observed in asthma are also induced by ozone. The mechanisms responsible for the greater increase in BHR observed in persons with more severe asthma may also augment the ozone-allergen interaction. Thus the quantitative and qualitative risks that are unique to persons with moderate or severe asthma exposed to ozone remains to be defined.

Another drawback of these studies is that they emphasize the effect of ozone on subsequent allergen challenge. It could be argued that increased exposure to elevated ambient ozone is an intermittent experience for most individuals with asthma. However, persistent exposure to indoor allergens, and the exacerbation of baseline airway inflammation, is a more common scenario. Such allergen-induced increases in inflammation arguably enhance nonspecific BHR and might enhance ozone-induced inflammatory changes as well. Thus it seems reasonable to examine the effect of inhaled allergen challenge on ozone-induced changes in spirometry, BHR, and inflammation.

Review of the published studies involving ozone exposure before allergen challenge in persons with mild asthma suggest that moderate-to-high exposures of ozone are required to consistently alter immediate reactivity to inhaled allergen. These findings are consistent with the epidemiologic observations of an increase in asthma

morbidity the day after high ambient ozone concentrations. Further assessment of the risk to persons with asthma posed by ozone will require additional studies to improve our understanding of the interactions between ozone and allergen on airway health.

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