Lung Function and Inflammatory Responses in Healthy Young Adults Exposed to 0.06 ppm Ozone for 6.6 Hours

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Rationale: **Exposure to ozone causes a decrease in spirometric lung function and an increase in airway inflammation in healthy young adults at concentrations as low as 0.08 ppm, close to the National Ambient Air Quality Standard for ground level ozone.**

Objectives: **To test whether airway effects occur below the current ozone standard and if they are more pronounced in potentially susceptible individuals, such as those deficient in the antioxidant gene glutathione S-transferase mu 1 (GSTM1).**

Methods: **Pulmonary function and subjective symptoms were measured in 59 healthy young adults (19–35 yr) immediately before and after exposure to 0.0 (clean air, CA) and 0.06 ppm ozone for 6.6 hours in a chamber while undergoing intermittent moderate exercise. The polymorphonuclear neutrophil (PMN) influx was measured in 24 subjects 16 to 18 hours postexposure.**

Measurements and Main Results: **Subjects experienced a significantly greater ($P = 0.008$) change in FEV_1 ($\pm$ SE) immediately after exposure to 0.06 ppm ozone compared with CA ($-1.71 \pm 0.50\%$ vs. $-0.002 \pm 0.46\%$). The decrement in FVC was also greater ($P = 0.02$) after ozone versus CA ($-2.32 \pm 0.41\%$ vs. $-1.13 \pm 0.34\%$). Similarly, changes in %PMN were greater after ozone ($54.0 \pm 4.6\%$) than CA ($38.3 \pm 3.7\%$) exposure ($P < 0.001$). Symptom scores were not different between ozone versus CA. There were no significant differences in changes in FEV_1, FVC, and %PMN between subjects with GSTM1-positive and GSTM1-null genotypes.**

Conclusions: **Exposure of healthy young adults to 0.06 ppm ozone for 6.6 hours causes a significant decrement of FEV_1 and an increase in neutrophilic inflammation in the airways. GSTM1 genotype alone appears to have no significant role in modifying the effects.**

Keywords: pulmonary function; airway inflammation; polymorphism; ozone exposure; exercise

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Inhalation of ozone causes decrements in lung function and an increase in airway inflammation at concentrations near the current Ambient Ozone Standard. It is not known what the effects are at concentrations lower than this.

What This Study Adds to the Field

This study reports that acute exposure to ozone for 6.6 hours at a level of 0.06 ppm (a level below the current Ambient Ozone Standard) causes significant effects on pulmonary function and airway inflammation in healthy young adults.

Ozone is a major component of photochemical smog. Controlled human exposure studies have been critical in demonstrating that it can cause decrements in lung function (1–7) and lung inflammation (8–11). Although the majority of these studies involved exposures to relatively high (0.1–0.4 ppm) concentrations for short periods of time (typically 2 h), prolonged exposure studies at lower levels were largely responsible for the U.S. Environmental Protection Agency (EPA) lowering the National Ambient Air Quality Standard for ground level ozone in 2008. Several studies have now confirmed the initial observation of Horstman and colleagues (4) that exposure of healthy young adults to 0.08 ppm ozone for 6.6 hours while undergoing intermittent moderate exercise will result in a significant drop (5–8%) in FEV_1 (5, 6). Similarly, studies using this exposure regime demonstrated that 0.08 ppm ozone can initiate inflammatory responses in the lungs of healthy young adults (9), characterized by increases in polymorphonuclear neutrophils (PMN) in bronchoalveolar lavage fluid collected 24 hours postexposure. Recently, we have confirmed and extended these findings in 15 healthy young adults by showing a significant increase in sputum neutrophilic inflammation 18 hours postexposure to 0.08 ppm ozone (12).

Recent community-based air pollution studies using emergency department records have reported associations for increased risk of pediatric asthma at very low ozone concentrations (13). This has led some to question whether adverse responses could occur in healthy adults at concentrations below the current standard. However, only two controlled human exposures have investigated this and none to our knowledge have addressed inflammatory effects. Adams reported that healthy young adults exposed to multiple ozone concentrations experienced a significant decrease in lung function at 0.08 ppm but not at 0.06 ppm or 0.04 ppm (7). However, a secondary analysis by others of the same data concluded that 0.06 ppm may have induced a change in FEV_1 (14). In 2009, Schelegle and colleagues (15) reported that healthy young volunteers exposed to step-wise ozone concentrations experienced a significant decrement of FEV_1 at concentrations as low as 0.07 ppm but not at 0.06 ppm.

Large heterogeneity in responses to ozone between individuals has been reported (16, 17). Ozone exerts oxidant stress and results in airway inflammation, and therefore genes that modulate inflammation and antioxidant defense mechanisms have been proposed as potential effect modifiers. In particular, glutathione S-transferase mu 1 (GSTM1) has attracted attention as it is present in the airways, and gene deletions (GSTM1-null genotype) can be present in up to 50% of the population,

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resulting in complete absence of the enzyme. This polymorphism has been associated with reduced lung function (18, 19), and numerous epidemiology and controlled exposure studies have suggested that it may confer susceptibility to increased airway inflammation to ozone as well as other oxidant air pollutants (20, 21).

More than 100 million people in the United States now live in the counties that do not meet the current ozone standard, and public health consequences are enormous. In the present study, we performed a randomized controlled human exposure crossover study and investigated specifically if exposure of healthy young adults to 0.06 ppm ozone for 6.6 hours with exercise would cause measurable airway effects assessed by pulmonary function (chosen *a priori* to be FEV_1 and FVC) and inflammatory markers (sputum PMN). We also examined if responses were more pronounced in individuals with the GSTM1-null genotype. Some of the preliminary results of these studies have been previously reported in the form of abstracts (22).

METHODS

Subjects

Fifty-nine healthy young adult subjects aged 19 to 35 years, with no history of smoking in the past 2 years, completed the study. All subjects underwent a screening procedure that included a complete medical history and physical examination and a pregnancy test for females. Exclusion criteria included respiratory illness or symptoms within 4 weeks or a positive pregnancy test. All subjects were genotyped for GSTM1. The study protocol was approved by the Institutional Review Board at the University of North Carolina Medical School in Chapel Hill and the EPA, and informed consent was obtained from all subjects before their participation in the study. Subject characteristics and baseline lung function test values are shown in Table 1.

Study Design

The study design was similar to those used in previous studies (4, 7) to facilitate comparison. Each subject was exposed to 0.00 ppm (clean air, CA) and 0.06 ppm ozone for 6.6 hours with moderate exercise in a stainless steel chamber ($4 \times 6 \times 3.2$ m). Exposures were randomized, double-blinded, and separated by at least 1 week. Minute ventilation (V_E) was measured hourly and exercise levels adjusted to $V_E = 20$ L/min/m^2 body surface area to ensure that subjects breathed consistently throughout exposure. Spirometric lung function and symptom scores were assessed immediately before and after the 6.6-hour exposure period. Sputum was collected the next morning approximately 16 to 18 hours postexposure. Exposures were conducted only during the cool weather season in Chapel Hill (November–March) to minimize exposure to elevated ambient ozone.

Study Protocol

Training day. On a training day, all subjects were trained for lung function measurements. Settings for the treadmill and bicycle ergometer required to produce a desired value of V_E was determined for each subject.

Exposure day. On the day of each exposure, subjects were assessed for vital signs, completed a symptom questionnaire, and performed preexposure lung function spirometry and body plethysmography maneuvers. They then entered the chamber set for the appropriate condition (CA or 0.06 ppm ozone) and began exercising for a 50-minute period at 20 L/min/m^2 body surface area in V_E followed by a 10-minute rest period. The exercise session was repeated six times alternating between the treadmill and bicycle with V_E maintained within $\pm$ 2 L/min of the subject's target value. Subjects were also given 35 minutes to eat lunch. Thus, they remained in the chamber for a total of 6.6 hours, during which ventilatory parameters (V_E, V_T, breathing frequency) were measured and electrocardiac signals, heart rate, and blood oxygen saturation monitored continuously to ensure subject safety. Subjects were exposed in pairs (of the same sex). At the end of exposure, spirometric and plethysmographic lung function measurements were performed and a symptom questionnaire was obtained.

Follow-up day. On the next morning, subjects returned to the laboratory and sputum samples were collected.

Measurements

Spirometry was performed on a 10.2-L dry seal digital spirometer interfaced to a computer (SensorMedics Model 1022; SensorMedics; Palm Springs, CA). At least three sets of qualified data were obtained and the largest value selected for FEV_1 and FVC as per American Thoracic Society guidelines (23). Pulmonary function on all subjects was measured using one dedicated spirometer and by one certified pulmonary function technician to minimize variability. Specific airway resistance (sRaw) was assessed by body plethysmography (SensorMedics Model V6200) and the average of two highest values out of three to five measurements obtained. Measurements were performed before and immediately after exposure for use in endpoint analysis. Additional spirometry measurements were performed in the chamber during 10-minute rest periods primarily for monitoring subject safety and were not necessarily performed by the same technician.

Symptoms were assessed before and after 6.6-hour exposure. The subjects were asked to rate the severity of cough, pain on deep inspiration (PDI), shortness of breath (SOB), and throat irritation on a five-point scale ranging from 0 (none) to 4 (most severe). Subjects recorded the severity score directly on the computerized questionnaire. Total symptom severity was obtained by adding scores of all four symptoms.

GSTM1 genotypes were determined using real-time polymerase chain reaction as previously described by Gilliland and colleagues (24) from DNA isolated from white blood cells using the QIAamp DNA Mini Kit (Qiagen Inc., Valencia, CA).

Sputum Collection and Analysis

Sputum samples were obtained and processed by the method described by Alexis and colleagues (25). Briefly, subjects underwent sputum

TABLE 1. SUBJECT CHARACTERISTICS AND BASELINE LUNG FUNCTION MEASURES

	All Subjects	Males	Females	GSTM1-p	GSTM1-n
No.	59	27	32	30	29
Age, yr	25.0 (0.5)	26.1 (0.9)	24.0 (0.5)	24.9 (0.8)	25.1 (0.7)
Height, cm	171.2 (1.2)	178.9 (1.2)	164.7 (1.0)	172.2 (1.5)	170.2 (1.9)
Weight, kg	70.5 (1.8)	78.8 (2.3)	63.4 (2.1)	71.5 (2.5)	69.4 (2.6)
BSA, m^2	1.8 (0.03)	2.0 (0.03)	1.7 (0.03)	1.8 (0.04)	1.8 (0.04)
BMI, kg/m^2	23.8 (0.48)	24.5 (0.72)	23.3 (0.64)	23.9 (0.68)	23.8 (0.69)
FEV_1, L	4.02 (0.10)	4.64 (0.12)	3.49 (0.07)	4.14 (0.12)	3.89 (0.16)
FVC, L	4.83 (0.13)	5.69 (0.14)	4.10 (0.11)	4.94 (0.17)	4.72 (0.21)
FEV_1/FVC, %	84.1 (0.8)	82.0 (1.1)	85.8 (1.2)	84.6 (1.2)	83.5 (1.2)
$FEF_{25-75\%}$, L/s	4.22 (0.14)	4.64 (0.23)	3.87 (0.14	4.39 (0.21)	4.05 (0.18)
FEF_{max}, L/s	9.03 (0.25)	10.61 (0.28)	7.69 (0.17)	9.49 (0.35)	8.54 (0.34)

Definition of abbreviations: BMI = body mass index; BSA = body surface area; $FEF_{25-75\%}$ = forced expiratory flow between 25 and 75% of FVC; FEF_{max} = maximum forced expiratory flow; GSTM1-n = glutathione S-transferase mu 1 null; GSTM1-p = glutathione S-transferase mu 1 positive.

Values are mean ($\pm$ SEM)

induction with hypertonic saline. Acquired samples were subjected to plug selection and subsequent treatment with dithiothreitol. Cell viability and total cell counts were evaluated and differential cell counts examined (Hema-Stain3; Fisher Scientific). Acquired sputum samples considered acceptable for processing had a minimum of 75 mg of selected plug material, cell viability greater than 50%, and squamous epithelial cells less than 40%. All sample processing and slide preparations were performed on the same day of collection.

Ozone Generation and Monitoring

Ozone was generated by a silent electric discharge method (Model 502; Meckenheim, Bonn, Germany) and introduced into the chamber that was maintained at 22 ± 1.0°C and 40 ± 5% relative humidity. The concentration of ozone was continuously monitored using ultraviolet photometric analyzers (TECO Model 49; Thermo Scientific, Franklin, MA) that were periodically calibrated for ± 5% accuracy by NIST traceable ozone calibrator (TECO Model 49PS).

Data and Statistical Analyses

The lung function endpoints were expressed as percent changes from the preexposure (or baseline) values for each subject. Neutrophil content in the sputum samples was expressed as percent of total cell count (%PMN) and the measurements after each exposure were compared. Linear mixed-effects models with a subject-specific random intercept was used to test changes in response endpoints between clean air and ozone exposures at the group level to account for subject-level variability and repeated measures. The effect of GSTM1 and separately sex was examined using a two-factor mixed-effects model with repeated measures on a single factor, exposure and subject-level random effects. We report the magnitude and direction of the expected change along with its associated 95% equal two-tail confidence intervals. R statistical software (Version 2.10.1) was used for the analyses. α of 0.05 was used to determine statistical significance.

RESULTS

Exercise and Minute Ventilation

Means of six hourly measurements of V_E, V_T, breathing frequency, and heart rate during 6.6-hour exposure to CA and ozone are summarized in Table 2. Overall, there was no difference in both ventilation parameters and heart rates between CA and 0.06 ppm ozone exposure.

Exposure to 0.06 ppm Ozone Causes Decrements in Lung Function

The primary hypothesis tested in this study was that exposure to 0.06 ppm ozone would decrease FEV_1 and FVC after 6.6 hours. The results are summarized in Table 3. Exposure to ozone resulted in a 1.71 ± 0.50% (mean ± SEM) decrease in FEV_1 compared with virtually no change (0.002 ± 0.46%) after exposure to CA (Figure 1A). Thus, relative to CA, exposure to 0.06 ppm ozone for 6.6 hours resulted in a 1.71 ± 0.64% decrement in FEV_1 (P = 0.008). These decrements did not appear to be driven by a small subset of subjects (Figure 1B). Of the 59 individuals studied, only three subjects showed greater than 10% drop after ozone exposure. Similarly, FVC decreased by 2.32 ± 0.41% after ozone exposure versus 1.13 ± 0.34% after CA (Figure 1C). Ozone exposure thus caused a relative decrement of 1.19 ± 0.51% (P = 0.02). Again, individual response to ozone exposure was mostly within ± 5% change, as shown in Figure 1D. Changes in other lung function parameters (forced expiratory flow between 25% and 75% of FVC, maximum forced expiratory flow, and sRaw) were not significant.

A second aim of the study was to determine the role of GSTM1 in determining responses to ozone. Although both genotypes had decrements in FEV_1 after ozone exposure relative to air, changes were only statistically significant for GSTM1-positive subjects (Figure 2A). However, the difference in FEV_1 response between GSTM1-null and -positive subjects was not statistically significant (P = 0.72). Similarly, females had a significant decrement in CA-adjusted FEV_1 (1.93 ± 0.88%, P = 0.02), whereas males did not (1.45 ± 0.95%, P = 0.14), but the difference between sexes was not significant (P = 0.66). No differences between GSTM1-null versus -positive and males versus females were seen for FVC (Figure 2B).

Exposure to 0.06 ppm Ozone Causes Pulmonary Inflammation

This study is the first to examine ozone concentrations below the current standard to cause pulmonary inflammation. The results are summarized in Table 4. Graphic illustration in Figure 3A shows that ozone exposure alters the airway milieu as evidenced by increases in %PMN in induced sputum samples. After air exposure, %PMN averaged 38.3 ± 3.7%. In contrast, ozone-exposed samples averaged 54.0 ± 4.6%. Thus, relative to clean air, ozone exposure resulted in a 15.7 ± 3.1% increase in %PMN for the whole group (P < 0.002). Of the 24 subjects studied, all but 5 subjects showed an ozone-induced increase in %PMNs and 10 showed greater than or equal to 20% increase (Figure 3B).

Figure 4 shows a significant increase in ozone-induced %PMN for both GSTM1-null (20.0%; 95% CI, 11.0–29.0; P = 0.001) and GSTM1-positive subjects (11.3%; 95% CI, 2.3–20.3; P = 0.02). Those carrying the null allele had a stronger response (P = 0.001) than those carrying the positive allele (P = 0.02); however, the estimate of the modifying effect of GSTM1 did not reach significance (P = 0.17; also *see* Table 4). Both males (24.2%; 95% CI, 15.8–32.6; P = 0.001) and females (8.5%; 95% CI, 0.79–16.20; P = 0.03) had statistically significant increases in ozone-induced %PMNs. The modifying effect of sex was significant (P = 0.009). The changes in %PMN were not accompanied by changes in total cell numbers for the whole group or any subgroup after ozone versus air. Total cell counts in sputum samples were 5.05 (± 0.82) × 10^6 after CA and 6.93 (± 1.52) × 10^6 after ozone (P = not significant [NS] vs. CA) for the whole group.

Symptom Questionnaire

Of 56 subjects who had no symptoms at baseline, 20 subjects reported symptoms after either CA or 0.06 ppm ozone exposure. The most commonly reported symptom was throat irritation followed by shortness of breath, pain on deep inspiration, and cough. The mean (± SEM) total symptom score was 0.43 ± 0.11 for CA and 0.41 ± 0.11 for ozone (P = NS versus CA). For genotype subgroups, total symptom score was 0.40 ± 0.16 for CA and 0.47 ± 0.17 for ozone in GSTM1-positive subjects and 0.46 ± 0.16 for CA and 0.35 ± 0.13 for ozone in GSTM1-null subjects (P = NS versus CA for both groups). The score and nature of the symptoms were similar between CA and ozone exposures.

TABLE 2. BREATHING PARAMETERS AND HEART RATE OF SUBJECTS DURING 6.6-HOUR EXPOSURE

	V_E (*L/min*)	V_T (*L*)	F (min^{-1})	Heart Rate (min^{-1})
Clean air	37.1 (0.2)	1.38 (0.05)	29.1 (0.9)	128.4 (1.1)
Ozone	36.5 (0.2)	1.37 (0.05)	29.0 (0.9)	127.0 (1.1)

Definition of abbreviations: F = breathing frequency.

Values are mean (± SEM). Note that the target value of V_E = 20 L/min/m^2. BSA corresponds to unadjusted mean value of 36.5 L/min.

DISCUSSION

In 2008 the EPA revised National Ambient Air Quality Standard for ground level ozone down to 0.075 ppm (3-yr average of the

TABLE 3. LUNG FUNCTION RESPONSES TO 6.6-HOUR EXPOSURE TO CLEAN AIR AND 0.06 PPM OZONE IN HEALTHY YOUNG ADULTS

	All Subjects ($n = 59$)		Males ($n = 27$)		Females ($n = 32$)		GSTM1-p ($n = 30$)		GSTM1-n ($n = 29$)	
	%Δ	95% CI	%Δ	95% CI	%Δ	95% CI	%Δ	95% CI	%Δ	95% CI
FEV_1										
CA	−0.002 (0.46)	−0.9, 0.9	0.59 (0.53)	−0.5, 1.6	−0.51 (0.72)	−1.9, 0.9	−0.08 (0.46)	−1.0, 0.8	0.078 (0.82)	−1.5, 1.7
Ozone	−1.71 (0.50)	−2.7, −0.8	−0.86 (0.62)	−2.1, 0.4	−2.43 (0.74)	−4.0, −1.1	−2.07 (0.71)	−3.5, −0.7	−1.34 (0.69)	−2.8, −0.1
Ozone-CA	−1.71 (0.64)*	−3.0, −0.5	−1.45 (0.95)	−3.4, 0.5	−1.93 (0.88)*	−3.8, −0.3	−1.99 (0.90)*	−4.0, −0.2	−1.42 (0.92)	−3.4, 0.3
FVC										
CA	−1.13 (0.34)	−1.8, −0.5	−0.77 (0.33)	−1.4, −0.1	−1.44 (0.56)	−2.5, −0.3	−1.11 (0.49)	−2.1, −0.2	−1.15 (0.48)	−2.1, −0.2
Ozone	−2.32 (0.41)	−3.1, −1.5	−1.26 (0.52)	−2.3, −0.2	−3.22 (0.57)	−4.3, −2.1	−2.41 (0.52)	−3.4, −1.4	−2.23 (0.65)	−3.5, −1.0
Ozone-CA	1.19 (0.51)*	−2.2, −0.2	−0.49 (0.74)	−2.0, 1.0	−1.78 (0.68)*	−3.1, −0.4	−1.30 (0.73)	−2.7, 0.1	−1.07 (0.71)	−2.5, 0.4

Definition of abbreviations: CA = clean air; CI = confidence interval; GSTM1-n = glutathione S-transferase mu 1 null; GSTM1-p = glutathione S-transferase mu 1 positive.

%Δ indicates change relative to preexposure, mean (± SEM).

* $P < 0.05$.

fourth highest daily maximum 8-h average) (26). This was based primarily on controlled human chamber studies of FEV_1 changes postexposure after 6.6 hours. Inflammation of the airway, as well as toxicology and epidemiology studies, were used as supportive evidence. In this study, we demonstrate, using the same exposure approach, decrements in FEV_1 and FVC at a concentration (0.06 ppm) below the current standard. Furthermore, we demonstrate increased neutrophil airways inflammation at this low ozone concentration.

To date, two previous studies have investigated lung function at 0.06 ppm ozone using a 6.6-hour protocol; neither found statistically significant effects (7, 15) even though their observed effect size was greater than in our study. For example, they reported clean air–adjusted FEV_1 after 0.06 ppm ozone decrements of 3.52 and 2.86%, whereas we observed a smaller drop of 1.71%, yet our results were highly statistically significance ($P = 0.008$). Similarly, our reported change in FVC of 1.18% was statistically significant ($P = 0.02$), although in the range of the 3.16 and 0.45% reported previously (7, 15). The key difference between the previous and the current study is that although their studies were designed to compare multiple concentrations at multiple time points, ours was specifically designed to limit the need for multiple comparisons.

To accomplish this, we used several strategies to increase the statistical power and to minimize experimental variability. First, our analysis was focused on only one ozone concentration and

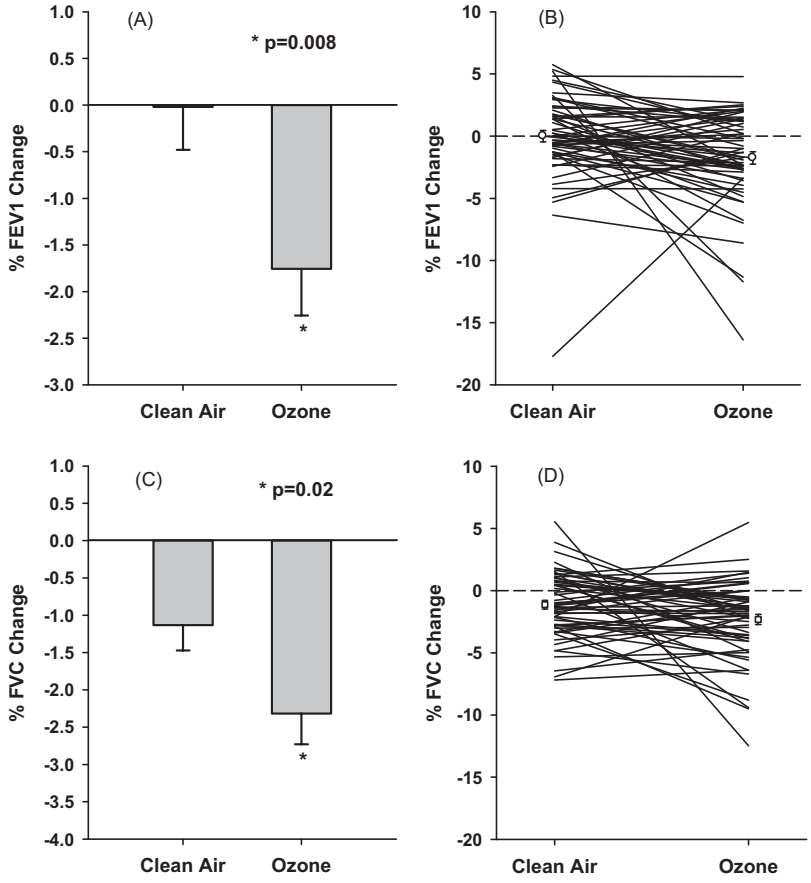

Figure 1. Percent changes in FEV_1 and FVC after 6.6-hour exposure to clean air and 0.06 ppm ozone. (*A*, *B*) Group means (± SEM) and individual changes, respectively, for FEV_1. (*C*, *D*) Group means (± SEM) and individual changes, respectively, for FVC.

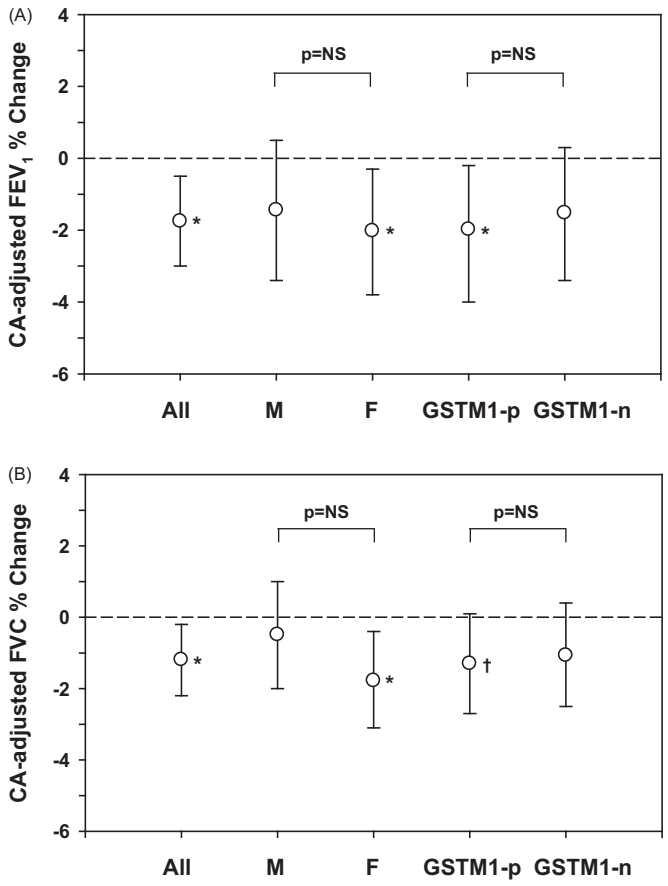

Figure 2. Clean air (CA) adjusted % changes (mean and 95% confidence interval) in (*A*) FEV_1 and (*B*) FVC after ozone exposure for all subjects, and by sex and GSTM1 genotype. *$P < 0.05$; †$0.05 < P < 0.1$. F = females; GSTM1-n = glutathione S-transferase mu 1 null; GSTM1-p = glutathione S-transferase mu 1 positive; M = males; NS = not significant.

a comparison to clean air. Second, we enrolled nearly twice as many subjects (n = 59) as previous studies, as the study was powered to examine the differences between two GSTM1 polymorphisms. Third, our analysis was determined *a priori* to focus only on changes observed immediately postexposure for lung function. Similarly, analysis of airway inflammation was limited to %PMN 16 to 18 hours after exposure. Fourth, we used only one dedicated pulmonary technician and spirometer for primary lung function measurements. Last, our studies were performed exclusively in the winter season, during which ambient ozone level was lower than 0.06 ppm (*see* Figure E1 in the online supplement), and thus potential influence of prior exposure to ambient ozone was minimized.

Our results may have significant public health implications. Although most subjects (> 60%) showed less than 10% decrease in lung function after 0.06 ppm ozone, 3 of 59 subjects in the present and 2 of 30 subjects in the Adams study (7) showed a lung function decrement greater than 10%. This suggests that 0.06 ppm ozone may cause lung function decrements in the majority of young individuals with some 6% (e.g., 5 of 89 subjects) of them having a greater response. In addition, this is the first study to examine and observe airway inflammatory effects for ozone at concentration levels below 0.08 ppm. Chronic airway inflammation may cause airway damage and thereby bronchoconstriction and bronchial hyperresponsiveness. An increase in neutrophilic inflammation has been shown to be strongly associated with exacerbation of airway disease in people with asthma. Thus, ozone is uniquely associated with worsening of asthma and increased hospitalization in children with asthma (13). Although the current study is limited to healthy subjects, the health consequences may be more severe in individuals with preexisting diseases, particularly in asthma.

Effects of sex on response to ozone exposure have been reported variably. Some studies found a greater response in FEV_1 in female than in male subjects, whereas others found a comparable response between them, mostly after a short-term exposure to high-concentration ozone (27, 28). For a prolonged exposure to low-concentration ozone, most studies report combined results of both males and females with no specific analysis for sex effects (6, 7, 15). The studies, however, appear to have assumed or found no sex effects in pulmonary function response. In our study, FEV_1 and FVC decrement was not significant in males but was significant in females; however, we found no significant difference between males and females. In contrast, %PMN was increased significantly in both sexes, with males showing a greater response than females ($P = 0.009$). Thus, it appears that ozone sensitivity may differ between sexes depending on endpoints.

A two-factor mixed-effects model with repeated measures was used to test if there was a difference in FEV_1 responses between GSTM1-positive and GSTM1-null subjects. We did not find such an association in either FEV_1 or FVC. This was consistent with earlier studies (20, 29) that have shown no independent role of GSTM1-null genotype on lung function decrement. The role of GSTM1-null, however, may become evident when it presents with other genotypes (NQO1 and GSTP1) (19, 29). Regarding airway neutrophilia, although the

TABLE 4. POLYMORPHONUCLEAR NEUTROPHIL RESPONSES TO 6.6-HOUR EXPOSURE TO CLEAN AIR AND 0.06 PPM OZONE IN HEALTHY YOUNG ADULTS

	All Subjects (*n = 24*)		Males (*n = 11*)		Females (*n = 13*)		GSTM1-p (*n = 12*)		GSTM1-n (*n = 12*)	
	Mean (± SEM)	95% CI	Mean (± SEM)	95% CI	Mean (± SEM)	95% CI	Mean (± SEM)	95% CI	Mean (± SEM)	95% CI
% Polymorphonuclear neutrophils										
CA	38.3 (3.7)	31.1, 45.6	33.4 (5.3)	23.0, 43.8	42.4 (5.1)	32.4, 52.4	34.7 (5.6)	23.7, 45.7	41.9 (4.8)	32.5, 51.3
Ozone	54.0 (4.6)	45.0, 63.0	57.6 (6.4)	45.1, 70.1	50.9 (6.6)	38.0, 63.8	46.1 (6.7)	33.0, 59.2	61.9 (5.5)	51.1, 72.7
Ozone-CA	15.7 (3.1)*	9.6, 21.8	24.2 (4.3)*	15.8, 32.6	8.5 (3.7)*	0.79, 16.2	11.3 (4.5)*	2.3, 20.3	20.0 (4.6)*	11.0, 29.0
Total cell count										
CA	5.05×10^6 (0.82)		4.08×10^6 (0.98)		5.86×10^6 (1.26)		3.97×10^6 (0.90)		6.13×10^6 (1.34)	
Ozone	6.93×10^6 (1.52)		9.42×10^6 (3.03)		4.83×10^6 (0.96)		5.58×10^6 (2.23)		8.28×10^6 (2.10)	

Definition of abbreviations: CA = clean air; CI = confidence interval; GSTM1-n = glutathione S-transferase mu 1 null; GSTM1-p = glutathione S-transferase mu 1 positive; PMN = polymorphonuclear neutrophils.

%PMN indicates polymorphonuclear neutrophils as % of total cell counts. SEM of total cell count is for the base number of 10^6.

* $P < 0.05$.

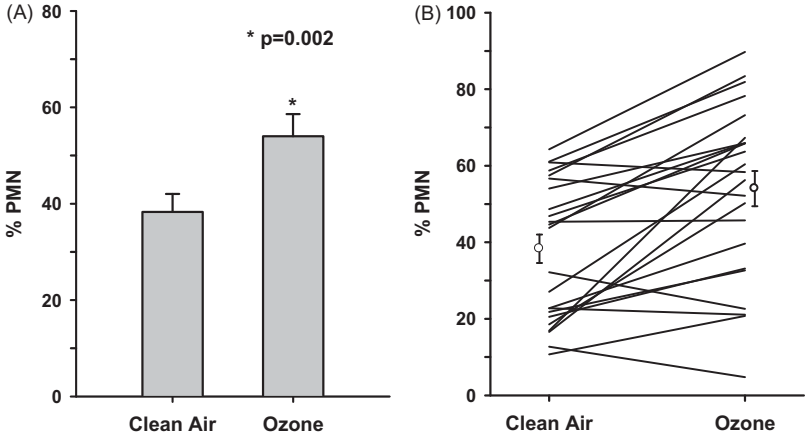

Figure 3. % Polymorphonuclear neutrophils (PMN) changes in sputum samples after 6.6-hour exposure to clean air (CA) and 0.06 ppm ozone for (*A*) the group mean, and (*B*) each individual. Error bars represent standard error. %PMN is defined by neutrophil number as % of total cell counts in the sample. F = females; GSTM1-n = glutathione S-transferase mu 1 null; GSTM1-p = glutathione S-transferase mu 1 positive; M = males; NS = not significant.

difference was not significant, GSTM1-null subjects appeared to have a greater neutrophilic inflammatory response than GSTM1-positive subjects. A sample size estimate, however, revealed that the nonsignificant increase would likely become significant with a larger cohort of approximately 47 subjects, which would be consistent with our earlier report on response of GSTM1-null genotype to 0.4 ppm ozone (20). This seemingly contradicting trend versus lung function response may be due to differences in the putative modes of action of ozone. Although changes in lung function are believed to occur via activation of a subset of airway C-fibers (30), inflammation is believed to originate from activation of nuclear factor-κB induced by reactive oxygen species generation (31). Thus, corticosteroids can blunt ozone-induced neutrophilia but not lung function responses (32). GSTM1 is believed to act by reducing oxidative stress, including detoxification of byproducts generated by inflammation. In line with previous studies (33), we found no relationship between lung function and inflammation.

In summary, our study shows ozone effects on two independent markers of airway health at a level as low as 0.06 ppm in healthy young adults. We did not find a significant role of GSTM1 genotype alone on the ozone-induced airway effects, but there may be individuals or subpopulations with enhanced sensitivity (such as in asthma) at these levels. We therefore conclude that exposure to ozone levels below the current standard can cause changes in lung function and initiate an airway inflammatory response in a young adult population and pose a health risk, particularly to susceptible populations.

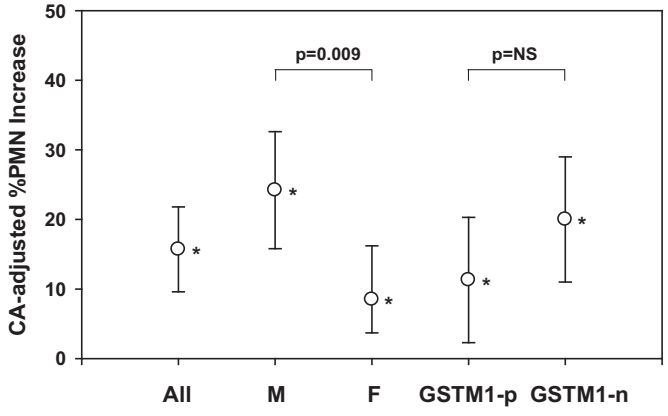

Figure 4. Clean air (CA) adjusted % changes in polymorphonuclear neutrophils (PMN) (mean and 95% confidence interval) after ozone exposure for all subjects, and by sex and GSTM1 genotype. *$P < 0.05$. F = females; GSTM1-n = glutathione S-transferase mu 1 null; GSTM1-p = glutathione S-transferase mu 1 positive; M = males; NS = not significant.

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