Distribution and reproducibility of spirometric response to ozone by gender and age

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Hazucha, Milan J., Lawrence J. Folinsbee,† and Philip A. Bromberg. Distribution and reproducibility of spirometric response to ozone by gender and age. J Appl Physiol 95: 1917–1925, 2003. First published July 18, 2003; 10.1152/japplphysiol.00490.2003.—Subjects were healthy nonsmoking men (n = 146) and women (n = 94) 18–60 yr old. Initially, each subject was exposed for 1.5 h to 0.42 ppm O₃. Forty-seven individuals were later reexposed twice, 1 wk to several months apart, to 0.4 ppm O₃. Intermittent exercise utilized in all exposures was adjusted to produce an O₃ dose of 560 ppm × m² body surface area. The post-O₃ percent change in forced-expiratory volume in 1 s (Δ%FEV₁) decrements of young (18–35 yr) and middle-aged (36–60 yr) men and women differed significantly (P < 0.05) from normal distribution with values skewed toward larger decrements in younger subjects. The mean Δ%FEV₁ rates were −16.3%, −16.6%, −11.6%, and −6.4%, respectively. The rate of decline with age was 2.5 times higher in young women compared with young men (P < 0.05). This pattern was reversed in the middle-age cohort. Our data support earlier reports of no significant difference in spirometric response to O₃ between young men and women. The data also confirm that large FEV₁ decrements after O₃ exposure are mostly confined to younger individuals that also show much greater variance in response to repeated exposures than the middle-aged subjects. The majority of subjects remained in their initial category of O₃ sensitivity on retesting after various time intervals (7). Although cross-sectional studies of middle-aged and older subjects have shown significantly lower average sensitivity to O₃ (assessed by spirometry) than observed in similarly exposed young cohorts (7), no longitudinal studies that could provide stronger evidence of age-dependent decline of an individual’s O₃ responsiveness, other than the study of Linn et al. (23) completed over a 2-year period, appear to have been performed.

As mentioned earlier, the interindividual variability of responsiveness to O₃ in young healthy individuals is substantial. Less is known about the interindividual variability among middle-aged (35–65 yr) individuals who comprise 38% of the United States population. The conventional approach to manage a broad spectrum of responses (Δ%FEV₁) such as that observed in young individuals has been to somewhat arbitrarily categorize subjects or responses as “weak,” “moderate,” and “strong.” The individual responsiveness to O₃ does not seem to be predictable from any indexes of lung function, including baseline nonspecific airway reactivity (9, 26). Attempts to identify a priori potentially responsive subpopulations or individuals were not successful. The intrapersonal variability of O₃ responsiveness, although generally lower than interindividual variability, differs substantially from individual to individual. Moreover, the variability increased as the elapsed time between O₃ exposures increased (25). Similar findings have been reported in preselected groups of exercising “weak” and “strong” responders to O₃ exposed at 2- to 3-mo intervals to 0.18 ppm O₃ at relative humidity and temperature simulating a smoggy Southern California day in the summer. The study has found substantial seasonal variations in mean FEV₁ response among initially “strong” responders (23).

HEALTHY ADULTS stratified by gender, age, and race when exposed acutely to O₃ show a significant interindividual variability in their symptomatic and lung function responses. Exposures of healthy young men for 2 h to 0.42 ppm O₃ with moderate (18) or heavy (24) intermittent exercise-induced forced expiratory volume in 1 s (FEV₁) decrements ranging from 2% to 48% of preexposure baseline. Healthy young women continuously exercising at a moderate load in 0.4 ppm O₃ for 1 h developed FEV₁ decrements from 0 to 41% (9). Although some studies (28, 33) suggest that young women are more responsive to O₃ than young men, the findings of gender differences are inconsistent.

Young individuals are on average the subjects that are the most responsive to acute O₃ challenges. A few studies of middle-aged and older subjects have shown significantly lower average sensitivity to O₃ (assessed by spirometry) than observed in similarly exposed young cohorts (7). Although cross-sectional studies have indeed shown that O₃-induced Δ%FEV₁ decreases with age, no longitudinal studies that could provide stronger evidence of age-dependent decline of an individual’s O₃ responsiveness, other than the study of Linn et al. (23) completed over a 2-year period, appear to have been performed.

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Although the above and other studies have shed some light on intra- and interindividual variations in sensitivity to O₃, the distribution of spirometric response to O₃ exposure in populations categorized by age, gender, or previous level of O₃ responsiveness has been explored in few studies only. Moreover, there is limited information available on gender and age-dependent rate of loss of O₃ sensitivity, gender differences and consistency of O₃ response in middle-aged individuals.

In the present analysis we have characterized distribution of postexposure FEV₁ (the most stable and representative spirometric endpoint of O₃ response) of the study population by gender, age, and level of O₃ responsiveness, and evaluated the differences, if any, between these cohorts. In addition, we have determined the rate of loss of FEV₁ with age for both men and women. We have also combined data from two similar exposure studies to retrospectively determine intraindividual consistency of response across three exposures completed over a 2-year period.

METHODS

The study protocol was approved by the School of Medicine Committee on the Protection of the Rights of Human Subjects of the University of North Carolina. The subjects for this study were healthy, at least moderately active nonsmoking male and female volunteers from the general population between the ages of 18 and 60 yr. The majority of subjects were students, faculty, and staff of the three major area universities, and the rest were individuals from the surrounding communities. Subjects underwent a screening procedure, which included Minnesota Multiphasic Personality Inventory, a family and personal medical history with an emphasis on the cardiovascular system, physical examination, spirometry, routine chemical screen (SMA20), and hematological (complete blood count with differential) tests; in addition, the women were screened with a urine pregnancy test. Allergy testing was performed by epicutaneous administration of 18 antigens common to central North Carolina. Potential subjects were excluded from the study if they had a history of allergic rhinitis, asthma, or chronic respiratory disease, heart disease, or regular exposure to dust or polluted environments. Subjects over 45 yr of age, with their informed consent, underwent a submaximal exercise test (modified Balke protocol with 3-min gradual cool-down period) to determine their tolerance to exercise and unmask latent cardiovascular abnormalities. Subjects who qualified for the study were trained in correctly performing pulmonary function tests and underwent exercise on a treadmill to determine a load that will elicit minute ventilation of ~33–45 l/min [20 l·min⁻¹·m⁻² body surface area (BSA)].

Each subject participated in one training session and one exposure session to 0.42 ppm O₃ at normal temperature (22°C) and humidity (40%). On arrival at the laboratory on exposure day, each subject had vital signs measured, and if a premenopausal woman, another pregnancy test. The subject was fitted with electrodes for a single-exercise ECG telemetry lead and then proceeded to the test room where he or she completed the symptom questionnaire and baseline pulmonary function testing. Afterward, he or she entered an exposure chamber and underwent experimental procedures as scheduled. All exposures were conducted in a 4 m × 6 m × 3.2 m stainless steel chamber. The chamber atmosphere was maintained by continuous air reconditioning and recirculation through high-efficiency particle filters. During O₃ exposures, nitric oxide, nitrogen dioxide, and sulfur dioxide concentrations were typically <0.02, 0.005, and 0.005 ppm, respectively. A detailed description of the exposure chambers was published by Glover et al. (13).

During a 1.5-h exposure, subjects alternated 20 min of exercise at a load determined earlier with 10 min of rest. The minute ventilation was monitored at the minute 3 and minute 17 for 2 min during the first exercise period and at minute 17 for 2 min during subsequent exercise periods, at the same time the exercise load was adjusted if necessary. During each exercise bout, a 10-s single-channel ECG strip was recorded at 5-min intervals. Depending on the post-O₃ spirometric response after the exposure to 0.42 ppm, each subject was classified either as a “weak” (FEV₁ change ≤ 5% of baseline), a “moderate” responder (5% < FEV₁ change < 15% of baseline), and a “strong” (FEV₁ change ≥ 15% of baseline). One to 25 mo later, a smaller cohort of subjects was reexposed to O₃ twice. The two follow-up exposures were 0.40 ppm O₃ with alternating periods of 15-min exercise at (17.5 l·min⁻¹·m⁻²BSA) and 15-min rest were separated by 1 to 61 wk. The cumulative O₃ dose of 560 ppm·l/m² (C × T × V) was equivalent among the three exposures. Routine spirometric testing with the use of a dry seal spirometer (CPI) or a spirometer (model 1085, Medical Graphics) was performed before and immediately after exposure.

The SAS statistical program (SAS Institute; Cary, NC) was used to calculate descriptive statistics for the variables of interest, with the key variable of FEV₁ expressed in the percentage of decline from preexposure value (100%). We calculated the distributions of response variables derived from spirometry as grouped by age, gender, and O₃ responsiveness. The statistics were calculated for data endpoints obtained after exposure and for differences between before and after exposure. The association of age, gender, and responsiveness with the response variables was analyzed with the Spearman rank correlation coefficient (rₛ). In this regard, the sample size of ~200 subjects is sufficient to detect statistical significance at the two-sided P ≤ 0.05 level for rₛ ≥ 0.15. Further assessment of the relationship between response variables and age, gender, and responsiveness was based on univariate, multiple regression, and general linear model methods. These analyses address the role of each of the respective demographic variables in a setting where the roles of the others are taken into account.

RESULTS

Effects of age and gender on O₃-induced distribution of response, cross-sectional analysis. Healthy male and female nonsmokers (n = 240), 18 to 60 yr of age, completed at least one exposure to O₃. Because most human O₃ studies have arbitrarily employed the age of 35 yr as a cut-off point for young population, we have classified the subjects for data analyses as “young” if 35 yrs of age and younger (n = 198), and “middle age” when older than 35 years (n = 42). Such classification should facilitate comparison of our data to those reported previously from other laboratories. After the first exposure, the mean post-O₃ decrement in FEV₁ was −16.3% (range +1 to −44%) for young men (n = 125; mean age 24.7 yr), −16.6% (range +2 to −53%) for young women (n = 73; mean age 24.3 yr), −11.6% (range 0 to −63%) for middle-aged men (n = 21; mean age 47.4 yr), and −6.4% (range +2 to −28%) for mid-
dle-aged women (n = 21; mean age 44.4 yr). The mean response of the latter cohort differed significantly (P < 0.05) from all other cohorts, and that of middle-aged men differed significantly (P < 0.05) from the two

Fig. 1. Box-and-whiskers plot for forced expiratory volume in 1 s (FEV₁) expressed as the percentage of mean decrement from baseline (100%) for the four cohorts. The box represents one interquartile range (25th–75th percentile), and the line inside the box is the median value. The end of each “whisker” extends up to 1.5 interquartile range. ●, Values between 1.5 and 3.0 interquartile ranges of the box; ○, outlier values.

Fig. 2. Normal probability plots of Studentized residuals of %FEV₁ for the four cohorts. Deviations of the points from a straight line indicate nonnormal distribution of the residuals consequent to unequal variance. Results are shown for young men (n = 125), young women (n = 73), middle-aged men (n = 20), and middle-aged women (n = 20).

younger cohorts. The box-and-whiskers plot for the four cohorts is shown in Fig. 1. Two values identified as outliers were omitted from subsequent analyses.

If categorized by responsiveness, 42 subjects (24 men, 18 women) qualified as “weak” (18%), 92 (57 men, 35 women) as “moderate” (39%), and 104 (64 men, 40 women) as “strong” (43%) responders. The middle-aged men were the only cohorts numerically evenly distributed among the three response categories. The young men and women were predominantly “strong” responders, whereas middle-aged women were mainly “weak” responders.

To test the distribution of these data for normality, we examined a normal probability plot of the Studentized residuals (Fig. 2). A formal test for normality showed that only the middle-aged men %FEV₁ responses were normally distributed. In the other three cohorts, the distribution of responses significantly deviated from a normal distribution (P < 0.05). A more liberal approach to data analysis, by potentially excluding several more extreme values (the Studentized residual value greater than ±2.0), did not influence the distribution curves in any significant way. Therefore, except for the two outliers identified in Fig. 1, we have kept these values in the data set since they represent responses of some highly responsive individuals.

The postexposure decrease in FEV₁ tended to be smaller with increasing age for both men and women. The quadratic model trend of postexposure %FEV₁ vs. age for both men and women was positive and significantly different from zero (P < 0.01 and P < 0.0001, respectively). The regression equation for men is %FEV₁ = 77.8 + 0.21 age + 0.001 age² (r = 0.242) and for women %FEV₁ = 59.3 + 1.23 age – 0.009 age² (r = 0.55).
Respective linear regression correlation coefficients are 0.242 and 0.488. To facilitate the utility of our data, we applied linear spline regression model to fit two straight lines for each gender group with knot (break point) at age 35. This age was selected because most O₃ studies have limited the age of their study subjects to ≤35 yr. The regression equation for men ≤35 yr is %FEV₁ = 79.1 + 0.19 age and for >35 yr is %FEV₁ = 72.2 + 0.385 age. For women, the respective equation is %FEV₁ = 66.2 + 0.71 age and %FEV₁ = 78.4 + 0.36 age. This analysis shows that the difference in the rate of loss of O₃ sensitivity between genders is driven by young women (Fig. 3). This was the cohort in which the loss of O₃ responsiveness with age was most substantial (0.71% FEV₁/yr) and statistically significant (P < 0.003). In young men, the rate was 0.19% FEV₁/yr, in middle-aged men 0.39% FEV₁/yr, and in middle-aged women 0.36% FEV₁/yr. The overall correlation by gender for the spline model was about the same as for the quadratic model, r = 0.246 for men and r = 0.495 for women, which indicates that the outcomes of the two models are nearly identical.

Reproducibility of O₃-induced response on repeated-exposures, longitudinal analysis. Of the 240 subjects, 47 (22 men, 25 women) underwent three exposures to O₃. The minimum and maximum number of elapsed days between the first and second exposure ranged from 27 to 737 days with a median value of 105 days, except for one subject who was reexposed for the second time 4 yr later. We have included this subject’s data in the analyses. The elapsed time between the second and third exposure was much shorter with a median value of 7 days.

Regardless of the age or gender classification, the mean baseline FEV₁ values (L) over the 3 exposure days were consistent and not significantly different.

However, for each response category (Fig. 4, A–C), the mean second and third postexposure %FEV₁ decrements were greater by 3 to 5 percentage points than the first postexposure mean decrements, respectively (i.e., the first O₃ exposure tended to produce smaller spirometric decrements than did the later exposures). The respective mean differences between the second and third exposures were <4 percentage points. A similar pattern of response was observed for percent forced vital capacity (not shown). Figure 4A shows the plots by gender. No significant differences were found between exposure days either when compared within or between the two gender cohorts. Figure 4B shows the data grouped by age category. Although no significant differences were observed between exposure days within cohorts, young individuals showed significantly
greater \((P > 0.05)\) changes on each of the exposure days than middle-aged individuals. Analysis of the age effect by gender classification showed significant differences \((P < 0.05)\) in the average \%FEV\(_1\) between young men or women and middle-aged men or women (plots not shown). Figure 4C plots the average \%FEV\(_1\) response for all subjects and by responsiveness categories over the three exposure days. Within pooled data (all subjects) as well as for each \(O_3\) responsiveness category (“weak,” “moderate,” and “strong”), there were no statistically significant differences between three exposures for either an absolute (a decrease, in ml) or a relative (%change) from a baseline \%FEV\(_1\). However, the group identified as “strong” responders on the first exposure day showed significantly greater decrements \((P < 0.001)\) at each exposure day than those identified on the first day as either “weak” or “moderate” responders. The corresponding day-to-day difference for the mean \%FEV\(_1\) between “weak” and “moderate” responders was significant \((P < 0.05)\) only for the first exposure day. Most of the “weak” responders were older individuals, whereas the “strong” responder group was dominated by young individuals.

The relation for individuals between their first-, second-, and third-day \%FEV\(_1\) responses is shown in Fig. 5. The regression lines between the first and second and the first and third day invariably fell below the line of identity, further indicating that the first exposure induced smaller effects than subsequent exposures (see also Fig. 4). The first vs. second and first vs. third day slopes were significantly different from each other \((P < 0.05)\) for every category, having respective correlation coefficient for men \((r = 0.850, 0.806)\), women \((r = 0.642, 0.695)\), young \((r = 0.723, 0.685)\), and middle-aged \((r = 0.696, 0.694)\) cohorts.

Figure 6 shows similar plots for initially “weak,” “moderate,” and “strong” responders. Because of a small number of subjects, no meaningful association can be calculated for the “weak” responders (Fig. 6A). The “moderate” and “strong” responders show similar association as other cohorts, with regression lines below the line of identity more so for “moderate” than “strong” responders. The slopes within each category were significantly different \((P < 0.01)\), and the correlation coefficients for both groups ranged from 0.616 to 0.544.

**DISCUSSION**

Over the past 30 years, over 30 \(O_3\)-exposure studies reported data obtained on mixed subject cohorts comprising 25% to 75% women. Three of the four studies (from the same laboratory) employing female cohorts reported only, that women were more responsive to \(O_3\), as assessed by changes in pulmonary function than comparable cohorts of men studied previously in their laboratory (11, 22, 28). The fourth study found no gender differences in \(O_3\) responsiveness between a cohort of women (19) and men studied earlier (6). Initially, the higher \(O_3\) responsiveness of women has been attributed to the smaller size of the female lung compared with the male one (22). A subsequent study from the same laboratory (28), however, found that lung size is not a determinant of a response. The investigators reported that for the same absolute minute ventilation the mean post-\(O_3\) decrements in \%FEV\(_1\) were about the same, regardless of lung size of the exposed women. Hormonal cycling influences studied in a group of young women seem to have only a minor effect on \(O_3\) responsiveness (33). More recently,
Housley and colleagues (20) suggested that the lower level of uric acid (antioxidant) in plasma and nasal mucosa of women might be a contributing factor to their greater responsiveness to O₃. Small volume O₃ bolus studies have demonstrated that gender differences in O₃ responsiveness can be fully accounted for by differences in anatomic dead space (4), which can in turn be directly related to BSA.

Only six studies were designed to systematically explore potential gender-based differences in lung function induced by O₃ exposure (1, 7, 12, 30, 31, 33). They all reported no gender-based statistically significant differences in effect of O₃ exposure on pulmonary function except for total airway resistance (Raw), which in one study (30) was significantly higher in women than men. The findings of our study with much larger cohort are consistent with absence of gender differences in responsiveness to O₃ exposure. Although young women responded (Δ%FEV₁) slightly more and middle-aged women slightly less than the respective male cohorts, these differences were not statistically significant.

Intrinsic susceptibility of individuals to O₃ has been demonstrated to be highly variable particularly among young individuals, ranging from no response to up to 50% decrements in FEV₁ after short-term exposures to ≤0.4 ppm O₃. We used a normal probability plot of the Studentized residuals to formally examine the normality of distribution. Despite an appearance of normality of our data on scatter plots, they were in fact not normally distributed. The S-shaped pattern of Studentized residual plots for young men and women indicate that the shape of the two distribution curves is symmetric and narrow but has long tails compared with a normal distribution curve. This is because the studied cohort had larger number of non- and “weak” responders on one tail and “strong” responders (including extreme responses) on the other tail of a distribution curve. Similarly, the shape of the residual scatter plots of middle-aged cohorts indicates that the distribution curves are skewed toward the side with the largest decrements. Although our study population was not randomly selected since we studied “self-selected” volunteers, our selection approach does not invalidate the obtained results. Because of nonnormal distribution of our data, we at first applied a second-order nonlinear regression function to it. Because the interpretation and utility of a nonlinear model is more complex than that of a linear model, we have used a linear spline regression model to derive two regression lines for each gender vs. age plot. As our results show, the spline model differs little from the quadratic model, simplifies the comparison of our findings with those published by other laboratories, and facilitates the usage of our model for prediction purposes.

On the basis of cross-sectional studies, the spirometric response (Δ%FEV₁) of young and middle-aged individuals exposed to O₃ has shown an age-dependent decline in average response. Cohorts of older men and women have responded much less intensely to O₃ exposure than did similarly exposed young subjects (8, 14). McDonnell and colleagues (27) reported ~53 ml/yr smaller decline in FEV₁ for men 18–32 yr of age intermittently exercising at 35 l·min⁻¹·m⁻² BSA during a 2-h exposure to 0.40 ppm O₃, on the average. We, however, found that a year-to-year post-O₃ FEV₁ will become smaller by only 17 ml/year when calculated for young men over the same age range. This is much lower rate than one would predict from McDonnell’s data even after adjustment for exposure time, which in our study was 25% shorter, and the exercise ventilation level, which was substantially lower (20 l·min⁻¹·m⁻² BSA) than at a slightly higher O₃ concentration (0.42 ppm). Indeed, the authors themselves found this “im-
provement” to be excessive because the extrapolated response (FEV<sub>1</sub>) would cross zero at age 39. Thus they suggested that the post-O₃ ΔFEV<sub>1</sub> vs. age relationship is most likely nonlinear and “discontinuous at some points beyond age 32.” This is indeed what we have observed and report in the present study. A comparison of the mean response in a study of Drechsler-Parks et al. (8) shows that FEV<sub>1</sub> decreased from baseline by 19.2% in a young cohort and by 5.6% in a cohort of old subjects after 2-h exposure to 0.45 ppm O₃ with intermittent exercise at a ventilation of 25 l/min. Our data, adjusted for the age range of Drechsler-Parks and colleagues study (8) show that FEV<sub>1</sub> decreased from preexposure by 16.7% in a cohort of young individuals and by 6.7% in a cohort of old individuals, on the average. Again, taking into account the differences in exposure protocol, the observed changes in the two studies are remarkably similar.

Several studies have reported that the intrinsic responsiveness of an individual to O₃ as assessed by spirometric endpoints is reproducible in young healthy men (25) and middle-aged asthmatic patients (17). However, healthy elderly (3) when reexposed 1 wk to 2 mo later showed rather poor reproducibility. These studies also reported that responsiveness varied greatly among different end points such as symptoms, spirometry, airway resistance, or inflammatory response. Of these, the spirometric variables, particularly FEV<sub>1</sub>, were considered to be the most reproducible because they showed the smallest difference between two separate exposures. The reported Pearson’s correlation coefficient (r) between the two exposures for FEV<sub>1</sub> was 0.98 and 0.77 for young individuals, 0.66 for middle-aged asthmatic patients, but only 0.47 and 0.33 for elderly subjects. Exposures that were separated by up to 13 mo showed r = 0.79 for young healthy individuals (25). The correlation coefficients in our study are in general agreement with the above studies and range from 0.85 to 0.69, depending on a cohort classification. However, for middle-aged individuals, we are reporting substantially higher r values than the ones reported for the elderly (3). As the authors pointed out, the data seem to show that the longer the interval between subsequent exposures, the greater the difference in responses, which holds true for our study as well.

The intersubject variability of response was greater than the intrasubject variability. In young and middle-aged subjects exposed on at least two separate occasions to O₃, the within-subject difference between respective post- to preexposure decrements in %FEV<sub>1</sub> for the two exposures, ranged from 0 (identical response) to −20 percentage points (estimated from the plots in Refs. 17 and 25). In older individuals the post- to preexposure Δ%FEV<sub>1</sub> ranged from 3 to −36 percentage points between subjects exposed on three different days separated by an average of 17 and 27 days, respectively. The mean Δ%FEV<sub>1</sub> differences were much tighter, however, and ranged from 2 to 6 percentage points (3). We have extended this observation by determining the reproducibility of spirometric response over three O₃ exposures separated by a wider time period at a comparable dose and exposure conditions. Regardless of categorization of our study cohort (men and women, young and middle aged, or “weak,” “moderate,” and “strong” responders) we have found that the mean FEV<sub>1</sub> response after the second exposure (median 105 days lag) was more pronounced (by 3–6% FEV<sub>1</sub>) though this was not statistically significantly different. Thus our findings for young and old subjects generally agree with published studies. A much shorter elapsed time between the second and third exposure was associated with more reproducible responses than between the first and second exposure, with average mean difference in %FEV<sub>1</sub> between exposures being less than ±2 percentage points. It is unclear why in the quasialongitudinal study of McDonnell et al. (25), the female cohort of Bedi et al. (3), and ours the second exposure produced greater FEV<sub>1</sub> decrements.

Although individual’s responsiveness may change from season to season, it remained consistent between years for the same season (23). We did not observe any season-associated patterns of intrinsic responsiveness in any of the cohorts. The number of first and second exposures was about evenly distributed between seasons. Although summer is the “ozone season” in our area, at the time of our studies our region did not reach nearly as high a concentration and frequency of high-O₃ days, as did Southern California, where the study of Linn et al. (23) was conducted. Between the first and second exposure several of our “weak” responders became “moderate” and several “moderate” responders became “strong.” Because our subjects were allergy skin-test negative, we do not think that some of the individuals became more responsive because of seasonal or other intermittent allergen exposure. Similarly, the data of McDonnell et al. (25) also acquired at different seasons of the year do not appear to be influenced by seasonal changes (but neither his nor our study was specifically designed to examine seasonal effects). The wide range of elapsed days between exposures in these four studies suggests that the pattern is not an exposure sequence effect, which has been demonstrated on repeated exposures done within 5 days. Although the subjects aged between the first and third exposure, considering the cross-sectional age-dependent annual attenuation of mean FEV<sub>1</sub> response, the FEV<sub>1</sub> loss for any cohort due to aging would amount to <1%, thus in any of these studies a negligible change. Collectively, these studies show that in an individual the postexposure %FEV<sub>1</sub> change on repeated exposures may be highly variable, reflecting changes in innate responsiveness (e.g., innervation of airways) confounded by age, gender, seasons, socioeconomic status, and exposure to other environmental agents.

O₃ exposure causes multiple effects on the airways. Spirometric changes are invariably accompanied by a modest increase in airway resistance, which is reflective of bronchoconstriction. Although we did not measure airway resistance, a negative though not equivalent association between spirometric variables (FEV<sub>1</sub>)
and Raw endpoints is well recognized. This is not surprising because both endpoints reflect many common pathophysiological mechanisms. Indeed, many human exposure studies have reported a post-O₃ decrease in FEV₁, with a small concomitant increase in Raw (15). Our recent study (10) further supports the evidence of minimal contribution of airway resistance to O₃-induced spirometric effects, reflecting insignificant increase in post-O₃ central inspiratory drive, which is known to modulate airway tone. Higher baseline airway resistance in older individuals (21) is unlikely a major modulating factor. Narrower airways should promote penetration of O₃ into smaller airways, which would be reflected in greater, not lesser, spirometric decrements.

The physiological mechanisms behind the extreme reaction of some individuals, large intrasubject variability, or age-dependent gender-differential rate of loss of O₃ responsiveness are unclear. We (29) have previously demonstrated that O₃-induced spirometric decrements (FEV₁) in healthy young and middle-aged adults are principally neural in origin, involving opioid-modulated sensory bronchial C-fibers. These peripheral nonmyelinated afferents are most likely the primary site of action, which would be compatible with a reflex action as well as a cortical mechanism. The findings also suggest activation of additional vagal fibers possibly involving rapidly adapting mechanoreceptors with A-δ afferents that are not opioid modulated (cough). Thus direct and indirect (possibly by PGE₂ stimulation and/or sensitization of opioid-modulated nerve fibers) appears to be largely responsible for most of the lung function decrements. Some recent studies of an opioid receptor system in humans reported significant age-associated decline in analgesic response to a painful stimulus (cold water immersion). Older individuals showed smaller response and required higher intensity of stimulus than younger subjects (32). Although the findings of pain perception studies are very dependent on the type of applied noxious stimulus, a change in sensitivity of opioid system by the aging process may be a plausible mechanism that may contribute to decreased responsiveness of older individuals to O₃.

In addition to the dominant nociceptive (opioid inhibitable) system, other mechanisms, such as nonspecific airway reactivity and inflammation, have been suggested as plausible contributing factors to the overall change in spirometric lung function, particularly in younger individuals (29). However, baseline nonspecific airway reactivity is not significantly different between men and women nor is it related to age (16). A similar lack of age-related differential in airway reactivity was reported for asthmatics that, in contrast to healthy individuals, are characterized by airway hyperreactivity (5). These studies suggest that the baseline airway reactivity will have minimal effects on O₃ responsiveness. How the intensity of O₃-induced airway inflammation in humans relates to age has not been studied. Human laboratory studies (2) have repeatedly reported a poor association between O₃-induced spirometric and inflammatory responses. Moreover, the peak inflammatory response develops well into the recovery phase of spirometric lung function; thus it is unlikely to influence it in a substantial way. Whether the marked inter- and intraindividual differences in response of healthy adults to O₃ inhalation can be attributed to differences in bronchial C-fiber anatomy and physiology or to differences in the inflammatory events after O₃ uptake and reaction with substrates in the airways lining liquid and epithelium is unknown at the present time.

Our results not only confirm but also extend many observations reported previously. The cross-sectional analysis shows that the loss of responsiveness to O₃ is gradual and by the age of 50 it begins to level off. We have also found that the rate of loss of response over the years is higher in women than in men. Young women lose their responsiveness three times as fast as young men, whereas during the middle-age period the rates are about the same. In our study, ~27% of young individuals experienced a >20% drop in FEV₁ and 12% > 30% drop in FEV₁, which may limit or even prevent more vigorous physical activity for several hours after exposure. Although the initial defense mechanisms are reflex in origin and usually resolve within hours, subsequent airway inflammatory changes (17) may take over 24 h to resolve. Thus it is not only impaired activity but also potentially higher risk of adverse response from concomitant or subsequent exposure to other pollutants and bioaerosols. Despite a progressive loss of sensitivity to O₃ with age, we found several apparently healthy middle-aged individuals who responded to O₃ exposure as strongly as young subjects. In these individuals, such decrements would be clinically significant and may induce other adverse health effects.

In summary, among healthy, self-selected, nonsmoking volunteers exposed to O₃, younger individuals show a much wider range and magnitude of acute response (as assessed by % decrement in FEV₁) than do middle-aged individuals exposed under similar conditions. With minute ventilation during exercise normalized for BSA, young men and women are on the average about equally responsive to a moderately intense exposure to O₃, though the responses significantly deviated from a normal distribution. “Strong” responses are less common over the age of 35 yr, especially in women. The rate of loss of responsiveness with age is highest in young adult women. The variability of an individual’s sensitivity to O₃ on repeated exposures decreases with age. Except for a few individuals, who showed a substantial shift between the responsiveness classes, particularly when the elapsed time between exposures was long, most individuals retained about the same intensity of responsiveness between exposures. This held true regardless of cohort categorization into females and males, young and middle-aged, and (initially) “weak,” “moderate,” and “strong” responders. Our data suggest that young individuals may be at a higher risk of adverse health effects when exposed to O₃ and
potentially more vulnerable to other noxious exposures.

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**DISCLOSURES**

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