Repetitive Ozone Exposure of Young Adults
Evidence of Persistent Small Airway Dysfunction

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Earlier, we found that acute ozone (O_3) exposure caused, along with inflammation, greater, more protracted changes in small airway function (isovolumetric $\dot{V}$max at intermediate to low lung volumes) than in FVC or FEV_1. To test if this distinction prevailed with repetitive O_3 exposure, we exposed eight healthy adults on four consecutive days alternatively to filtered air (FA) and O_3 (0.25 ppm $\times$ 2 h). Isovolumetric $FEF_{25–75}$, $\dot{V}$max50, and $\dot{V}$max75, were grouped into a single value representing small airway function (SAW_{grp}); respiratory frequency (f) and tidal volume (V_T) were monitored during exercise. On Day 5, peripheral airway resistance (Rp) was measured followed by lavage. All daily spirometric and ventilatory changes declined in magnitude (adapted) after one or more days of O_3 exposure. In addition, SAW_{grp}, f, and V_T showed persistent changes beginning with Day 2, denoted either by depression of the preexposure baseline (SAW_{grp}) or exaggerated tachypnea during exercise. O_3-induced neutrophilia ($p = 0.04$) was present in lavage fluid. The possible relationship between these persistent changes in small airway function, measured in days, and the likelihood of cumulative injury in the same region if exposure is long term, is unknown.

Keywords: adaptive response; persistent response

The use of repetitive daily exposure to assess the cumulative effects of ozone (O_3) has been a staple of clinical toxicology (1–6). Although virtually all these studies relied at least in part on spirometry to plot the time course of the functional response, none was specifically designed to assess small airways. The latter have long been a focus of clinical interest because of their likely role in the pathogenesis of chronic lung disease (7). Earlier experience in this laboratory suggested that O_3-induced changes in small airway function following acute exposure differed in magnitude and duration from those involving the larger central airways and the volume capacity of the lung. Whereas recovery of FVC and FEV_1 began within 30 min of the end of exposure and was complete within 24 h, small airway constriction, as measured by the reduction in isovolumetric (isoV). $FEF_{25–75}$, $\dot{V}$max50, and $\dot{V}$max75, continued to increase during the first 30 min postexposure and showed little or no evidence of remission 24 h later (8–10). Also, whereas the changes in FVC and FEV_1 were unimodal in distribution and skewed toward minimal responsiveness, the changes in (isoV) $FEF_{25–75}$ showed a wider distribution that may have constitute more than one mode (8). At 24 h postexposure, the reduction in (isoV) $FEF_{25–75}$ correlated significantly with the increase in the fibrinogen level in bronchoalveolar lavage fluid (BALF), used as a marker of plasma fluid exudation (10).

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In testing the hypothesis that repetitive exposure to the same inhaled dose of O_3 over a 4-d period, as might occur during an episode of photochemical smog, would affect small airways disproportionately, we compared the results among both spirometric and ventilatory variables (respiratory frequency [f] and tidal volume [V_T]) for evidence of adaptive and persistent responses. As is customary, we have defined an adaptive response as one characterized by a diminution in the daily effect of O_3 with repeated exposure and a persistent response as one characterized by either a depression of the baseline (spirometry) or a mean change in ventilatory pattern (f, V_T) in the days following the initial exposure.

METHODS

Subject Selection

We studied eight healthy young adults (five white males, two white and one black female), 25 to 31 yr of age. Four subjects (Numbers 1, 4, 5, and 6, Table 1) had participated in O_3 studies one or more years earlier. None of the subjects smoked. We defined nonsmoking as equivalent to a lifetime total of less than 3 pack-years plus abstinence from smoking for at least 1 yr prior to the study. All underwent a screening procedure consisting of a medical history, physical examination, spirometry and electrocardiogram (ECG) (12 leads) during rest, and submaximal exercise on a treadmill (Model 20-55; Quinton, Inc., Seattle, WA). Minute ventilation ($\dot{V}_E$) was measured throughout the exercise test. Subjects were excluded from the study if they had a history of chronic respiratory or cardiovascular disease, upper respiratory infection during the past 6 wk, FVC, or FEV_1 values $\leq$ 80% predicted, or showed an apparent inability to sustain moderately heavy exercise for at least 30 min. Information on the women's menstrual cycle was recorded but not considered during scheduling based on our previous findings that the cycle did not affect O_3 responsiveness (9). The study and consent forms were approved by the Committee on Human Volunteers of the Johns Hopkins School of Hygiene and Public Health; informed consent was obtained from each subject.

Experimental Procedure

The study required 11 visits by each participant: once for the screening procedure and five times for each of the two regimes, O_3 and filtered air (FA), that is, four consecutive days of chamber exposure followed 24 h later by bronchoscopy. The sequence of the two regimes was randomized. Participants in previous O_3 studies were exempted from the screening procedure. Three or more weeks separated the O_3 and FA regimes. All subjects who began the study completed it. A single exceedance of the 1 h ambient O_3 standard occurred during the week that subject 5 was administered FA; he was cautioned to minimize outdoor activities. Prior to exposure, subjects were instructed to withhold vitamin supplements for 3 d and avoid caffeine-containing beverages on the morning of exposure. The exposures lasted 130 min. The first 120 min consisted of alternating 30-min periods of rest and exercise. The exposures ended with a final 10-min rest period.

The exercise level was set by controlling the speed and inclination of the treadmill. The level selected for each subject was based on the results of the screening submaximal exercise test. Our objective was to induce a level of $\dot{V}_E$ equivalent to about 8 $\times$ FVC, thereby normalizing exposure to an index of lung size. $\dot{V}_E$ was measured during the 8th, 18th, and 28th min of each exercise period with the subject wearing a nose clip and breathing through a one-way valve connected to a dry-gas meter. Respiratory frequency (f) was counted visually from

TABLE 1. PHYSICAL CHARACTERISTICS, BASELINE PULMONARY FUNCTION

Subject No.	Sex	Race*	Age (*yr*)	Height (*cm*)	Weight (*kg*)	FVC *L* (*%pred*)	FEV_1 *L* (*%pred*)
1	F	C	31	170.2	61.2	4.14 (108)	3.00 (94)
2	F	C	29	165.1	64.0	4.08 (103)	3.25 (108)
3	F	B	28	154.9	86.6	4.06 (143)	3.26 (135)
4	M	C	25	172.7	65.8	4.98 (100)	4.20 (102)
5	M	C	27	175.3	80.7	4.71 (92)	3.79 (91)
6	M	C	26	186.7	98.4	6.02 (102)	4.78 (100)
7	M	C	29	180.3	77.1	5.77 (106)	4.65 (106)
8	M	C	26	190.5	81.2	5.73 (93)	5.17 (103)

* C = white; B = black.

the dry-gas meter gauge; average tidal volume (V_T) was calculated from $\dot{V}_E$ and f. Chamber temperature was recorded during the procedure and used to convert $\dot{V}_E$ to BTPS. If necessary, the work load was adjusted to maintain the desired $\dot{V}_E$. The procedure was intended to control $\dot{V}_E$ and allow adjustments in V_T and f.

Heart rate was monitored electronically throughout exposure (Model Exersentry 3A; Computer Instruments Corp., Monroeville, PA), and arterial oxyhemoglobin saturation (Sa_{O_2}) was monitored with pulse oximetry (Nellcor-200; Nellcor, Hayward, CA) during the rest period only.

$FEF_{25–75}$, $\dot{V}$max50, and $\dot{V}$max75 were used to assess changes in small airway caliber. All values following exposure were adjusted for any changes in FVC that occurred, so that the before–after comparisons were isovolumetric. Our method of adjusting volume from the spirometric tracing and the assumptions underlying the method were described earlier (8, 11).

Subjects performed spirometry three times: before entering the exposure chamber (control), just before leaving the chamber (endexposure), and 25 min after leaving the chamber (25 min postexposure); all postexposure values reported are means of measurements made at endexposure and 25 min postexposure. On Day 5, the subjects underwent bronchoscopy for the measurement of peripheral airway resistance (Rp) and for bronchoalveolar lavage. Spirometry was measured in the bronchoscopy suite before and after bronchoscopy.

Spirograms were obtained in triplicate in accordance with the recommendations of the American Thoracic Society. The measurements were made with an 8-L water-sealed spirometer (Warren Collins, Inc., Braintree, MA) provided with a potentiometer connected by an analog digital converter to an IBM PC. The spirometer was calibrated daily and temperature was recorded before each set of measurements. The spirograms were reviewed visually for quality; any marred by coughing were eliminated. All spirometric variables were temperature corrected automatically and calculated using a software program developed locally. The forced expiratory effort producing the highest sum of FVC + FEV_1 is reported. For the maximum flow-volume curves, the volume signal from the potentiometer was differentiated electronically to give flow; both signals were recorded using the same software.

Prior to bronchoscopy on Day 5, subjects inhaled 5 ml of aerosolized 4% lidocaine (DeVilbiss nebulizer no. 646, Somerset, PA) and had a balloon-tipped catheter passed by nose to the lower esophagus to measure local pressure; the latter was used as a surrogate for pleural pressure. Subjects were than placed supine and trained to breath-hold at their functional residual capacity (FRC) after three deep breaths; reproducibility of FRC during breath-holding was checked by esophageal pressure. All subjects were premedicated with 100 μg of fentanyl given intravenously. Additional topical anesthesia was achieved by instilling 2% lidocaine through the bronchoscope. Four randomly chosen subjects also received 0.6 mg of atropine intravenously. Before the bronchoscope was wedged, the working channel was cleaned with a brush and a #5 FR double-lumen catheter was inserted. The bronchoscope was wedged into the anterior segment of the right upper lobe, with care taken to return to the same site on each subject. Five percent CO_2 in air was administered at a rate determined by a mass flow controller (Sierra, Carmel Valley, CA) through one lumen of the catheter ($\dot{V}_B$) while pressure at the tip of the bronchoscope (P_B) was measured through the other lumen. $\dot{V}_B$ was increased incrementally from 200 ml/min to 500 ml/min or until P_B reached 16 cm H_2O, then decreased to 100 ml/min and finally returned to 200 ml/min. Each flow was maintained until P_B had stabilized (approximately 5 to 15 s); the subject was then instructed to take three deep breaths and breath-hold at FRC after the third breath, while the steady-state P_B was recorded. The breath-holding maneuver was repeated at least twice at each $\dot{V}_B$. Rp was calculated as the average $P_B/\dot{V}_B$ at each static flow and averaged over five flows. The measurement has also been referred to as collateral resistance (R_{coll}) (12, 13).

After Rp was measured, the bronchoscope was withdrawn to the trachea, additional 2% topical lidocaine was given topically, if needed, and the bronchoscope was repositioned in the right middle lobe. The lobe was lavaged with five aliquots (20 ml each) of normal saline warmed to 37° C. The returns were combined and kept on ice until the end of the procedure, when small amounts were removed for determination of cell counts, cell viability, and differential staining. The samples were then centrifuged at 600 × *g* for 15 min at 20° C and the supernatants subdivided for measurement of albumin, fibrinogen, and kinins.

The techniques for the differential cell counts and biochemical assays were described previously (10). The kinin assay does not distinguish between bradykinin and lysylbradykinin on a molar basis.

Environmental Chamber

The details of the exposure system were described previously (10). A free-standing chamber measuring 2.7 × 2.1 × 2.4 m, with 10-cm-thick insulated sides, floor, and ceiling and lined on the interior with anodized aluminum, was used for exposure. The chamber could accommodate two subjects and was furnished with a spirometer, chairs, and a treadmill controlled from outside (Model 20-55, Quinton, Inc., Seattle, WA). Outdoor air was supplied to the chamber air-purifier through the central air-conditioning system that filtered coarse particulate matter and controlled air temperature and relative humidity thermostatically. The chamber flow rate was 5.6 m^3/min (23.7 air changes/h, one-pass design). Static pressure within the chamber was maintained slightly below (−0.1 cm H_2O) that of the surrounding laboratory by adjustment of the supply damper. The mean chamber temperature and relative humidity were 21.4 ± 0.2 (SD)° C and 46.3 ± 3.5% during FA treatment, and 21.4 ± 0.2 and 43.9 ± 2.4% during O_3 treatment.

Ozone was generated by electrical arcing (Model G1-L Ozone Generator, PCI Ozone Corp., West Caldwell, NJ) of 100% oxygen and mixed with purified air entering the chamber. The concentration of O_3 in the chamber was monitored with an ultraviolet photometer (Dasibi 1003 AH; Glendale, CA) at two sites near the breathing zones for the treadmill and chairs. All tubing to the monitor was Teflon; the monitoring line valves were stainless steel. O_3 monitoring was continuous during both FA and O_3 exposures and was recorded every minute. The concentration per exposure was based on the mean of five evenly spaced measurements made every 20 min after the first 5 min. The mean (± SD) concentration during O_3 exposure was 0.254 ± 0.001 ppm, and during FA exposure was 0.003 ± 0.001 ppm.

Statistical Analysis

We used the principal components statistical method (14) to group the three isovolumetric spirometric measures of small airway function ($FEF_{25–75}$, $\dot{V}$max50, $\dot{V}$max75), thereby forming a single value for each subject. The symbol used in the text to designate the value is SAW_{grp}. Our rationale for selecting this method along figures showing the results for the individual components of SAW_{grp} is presented in the Appendix.

Analysis of longitudinal data. Because repeated measures on the same subject violate the assumption of independence in linear regression, we used a "mixed effects" (combined fixed and random effects) regression model to analyze the longitudinal data on spirometric and ventilatory variables:

$$\text{Outcome} = \beta_0 + \beta_1{*}O_3 + \beta_2{*}\text{day} + \beta_3{*}O_3{*}\text{ day} + b_1{*}\text{ subject}$$

where β_0 was the intercept, the explanatory variables included O_3 treatment modeled as a binary variable, day of exposure as a categorical variable, O_3*day as an interaction, and b_1* subject, the study population, as the random effect (15). The analyses were performed on un-

transformed data and included the following: differences between FA and O_3 treatments: a, overall regression coefficients; b, paired points; differences within each treatment: c, daily pre- to postexposure changes to assess adaptive responses; d, day-to-day changes in baseline (spirometry) or mean value (f and VT during exercise) to assess persistent responses. Significant changes in the components of the model are cited in the figures. Significance was taken as $p < 0.05$.

RESULTS

During exercise, overall $\dot{V}_E$ averaged 38.6 ± 2.5 (SEM) L/min on FA and 39.1 ± 2.1 L/min on O_3. The mean values on individual days ranged between 38.1 ± 2.8 L/min and 39.1 ± 2.6 L/min on FA, and between 38.1 ± 2.1 L/min and 39.8 ± 2.2 L/min on O_3. No significant trends were noted over the 4-d periods. The overall targeted $\dot{V}_E$ was 39.5 ± 2.3 L/min.

Spirometry

The coefficients of variation for all spirometric variables including the small airway group (SAW_{grp}) are shown in Table 2; the values were based on FA breathing. Similar gradients in measurement error have been reported before (11, 16). The coefficient of variation was highest among the small airway variables, tending to increase as lung volume and flow rate fell. Such large coefficients clearly limit the utility of these measurements for detecting functional change.

The time course of the adaptive response differed slightly among spirometric variables (Figure 1). The maximal mean reductions in FVC and FEV_1 occurred on Day 2 of exposure to O_3; −7.7 ± 4.5% (SEM) ($p < 0.01$, model components b, c, and d) and −9.1 ± 5.7% ($p < 0.01$, model components b, c, and d), respectively. By Day 4, these daily changes were negligible. The maximal mean reduction in the SAW_{grp} occurred on Day 1 of exposure, −6.7% ($p < 0.05$, component b), and thereafter dissipated rapidly.

The only spirometric variable to show a persistent response with repetitive O_3 exposure was the SAW_{grp} (Figure 2). By Day 2, the SAW_{grp} baseline was depressed by 6.9 ± 3.5% ($p = 0.06$, component c) and remained depressed by slightly more than 8% ($p < 0.05$, component c) over the next 3 d. This persistent effect exceeded in magnitude the maximal pre- to postexposure reduction in the same variable seen on Day 1 (Figure 1).

Breathing Pattern

To test for evidence of an adaptive response affecting the pattern of breathing, we compared the magnitude of within-day changes in f and VT across the 4 d of exposure. The changes occurred between the 10th and 60th minutes of exercise, that is, between the first and last of six measurements made during exercise (Figure 3).

TABLE 2. WITHIN-SUBJECT MEASUREMENT VARIABILITY: COEFFICIENT OF VARIATION (CV)*

	Mean	Variance	CV (%)
FVC, L	4.95	0.012	2
FEV_1, L	4.05	0.011	3
FEF_{25-75}, L/s	3.38	0.054	7
$\dot{V}$max50, L/s	3.97	0.093	8
$\dot{V}$max75, L/s	1.59	0.053	14
SAW_{grp}†	1.00	0.012	11

* Based on preexposure measurements (eight subjects) on filtered air days, adjusted for repeated measures on the same subject; calculated as variance/mean ×100.

† SAW_{grp} = small airways group consisting of FEF_{25-75}, $\dot{V}$max50, and $\dot{V}$max75.

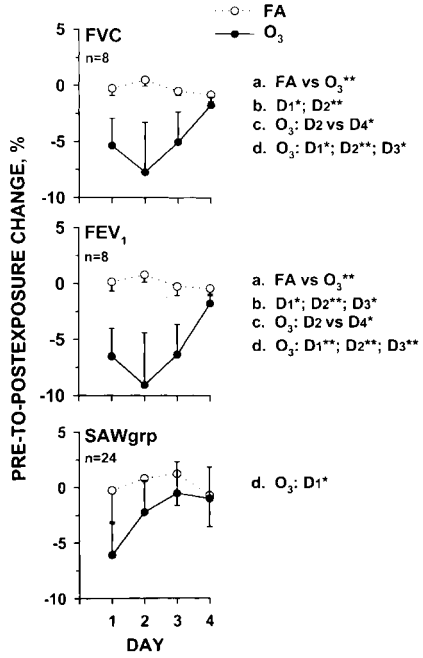

Figure 1. Adaptive response to repeated O_3 exposure. Mean, SEM. Components of the mixed effects statistical model include (1) the differences between filtered air and ozone: a, overall; b, paired points; and (2) the differences within filtered air and ozone: c, day to day; d, within day. *$p < 0.05$, **$p < 0.01$; FA = filtered air; O_3 = ozone; D = day.

The maximal mean increase in f during O_3 exposure occurred on Day 2, +6.6 ± 2.7 (SEM) breaths/min (BPM) or +30% ($p < 0.01$, component d). By Day 4, the change was indistinguishable from that seen with FA. (Respiratory frequency tended to increase slightly over the course of exercise on FA. On Day 4, this increase was similar for FA and O_3 although absolute f was higher with O_3.) The maximal mean reduction in VT during O_3 exposure occurred on Day 3, −0.36 ± 0.09 L or −19% ($p < 0.01$, component d). Evidence of an adaptive response appeared the next day: −0.22 ± 0.08 L or −11% ($p < 0.05$, component d).

To test for evidence of persistent changes in breathing pattern attributable to O_3, we compared the mean values for f and VT on Day 1 (based on all six measurements) against the means on the remaining 3 d (Figure 4). In effect, the pattern of breathing on the first day of exposure constituted the "baseline." (The means on Day 1 were similar for FA and O_3. Either could have served as baseline and yielded the same outcome. We selected the O_3 values to conform to the analysis carried out on the spirometric variables, as in Figure 2.)

On Day 2 of O_3 exposure, mean f was elevated by +4.7 ± 2.7 BPM or +22% ($p < 0.01$, component c), then gradually tapered off to +3.2 ± 1.4 BPM or +15% by Day 4 ($p = 0.06$, component c). Mean VT was reduced by −0.2 ± 0.1 L or −14% ($p < 0.01$, component c) on Day 2 and remained at essentially this level thereafter. This persistent pattern of more rapid, shallow breathing is foreshadowed in Figure 3: note that beginning with Day 2, the first of the six measurements of f and VT were at new levels compared with Day 1, further evidence of "carryover" effects on the control of breathing.

BALF Content

The total cell count (Day 5) tended to be higher after O_3 than FA, but the difference was not significant ($p = 0.26$ [Table 3]).

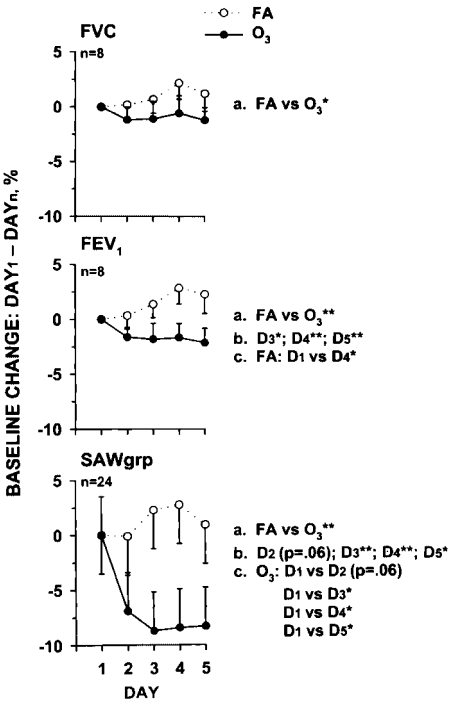

Figure 2. Persistent effect of repeated O_3 exposure on small airway function (SAW_{grp}). Mean, SEM. See legend to Figure 1 for description of components of the statistical model. FVC and FEV_1 baselines were not significantly depressed by O_3 (model component c). The small but significant differences between FA and O_3 for both FVC and FEV_1 (model components a and b) were due in great measure to the unexplained upward shift in their baselines over the course of FA breathing. *$p < 0.05$; **$p < 0.01$; FA = filtered air; O_3 = ozone; D = day.

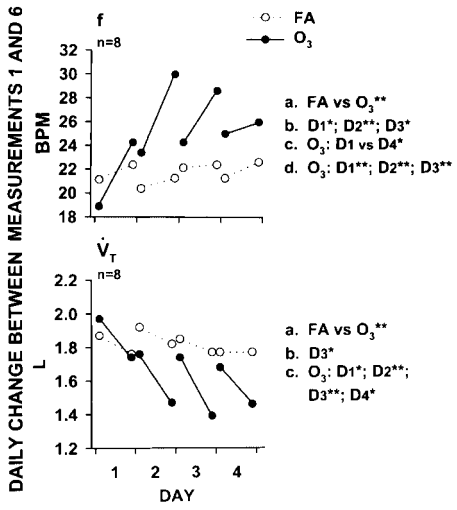

Figure 3. Adaptive changes in respiratory frequency (f) and tidal volume (V_T) during exercise. Mean, SEM. See legend to Figure 1 for description of components of the statistical model. Mean ventilation (L/min) was maintained at about 8 × FVC per subject. The values shown are the first and last of six measurements made at 10 min intervals during 1 h of intermittent exercise. The SEMs of these mean within-day changes on Days 1–4 were as follows: for f on FA (1.0, 0.9, 1.0, and 0.8 BPM) and on O_3 (1.4, 2.7, 1.7, and 1.4 BPM); for V_T, the SEMs were 0.1 L on both treatments, all days. *$p < 0.05$; **$p < 0.01$; BPM = breaths/min; L = liters.

The neutrophilic (polymorphonuclear, PMN) count was significantly higher after O_3: + 61% ($p = 0.04$). None of the cell types, expressed as percentages of total cells, differed significantly in the two circumstances.

The mean levels of albumin, fibrinogen, and kinins were not significantly different after O_3 and FA. Nonetheless, these findings were notable in two respects: O_3–FA differences in all three variables were strongly interdependent within subjects and varied widely in magnitude among subjects. Thus, a, the correlation coefficients (r) for the O_3–FA differences among the three variables, ranged between 0.84 and 0.93 ($p < 0.01$); b, the percentage increases in albumin, fibrinogen, and kinins in the two subjects with the most marked exudative responses, exceeded the mean changes for the group, respectively, by 10-fold, 15-fold, and 2-fold (Subject 2), and by 45-fold, 17-fold, and 7-fold (Subject 4).

Correlations between the changes in exudative markers and PMN counts were negligible (r = 0.05 to 0.20). Correlations between the changes in both exudative markers of inflammation and PMN counts and the level of depression of the small airway baseline ($SAW_{factor1}$; see Appendix for derivation) on Day 5 were inverse and weak (r = −0.30 to −0.46); the highest value, −0.46 ($p = 0.26$), was found between the changes in fibrinogen and $SAW_{factor1}$). Correlations between the changes in PMN counts and changes in both f and V_T on Day 5 were moderately strong (r = −0.52 and 0.65, respectively), but appeared paradoxical, that is, as PMN influx increased, the degree of rapid shallow breathing decreased. A similar paradoxical association between the degree of neutrophilia and the decline in FEV_1 has been seen following acute O_3 exposure (17, 18).

Peripheral Airways Resistance

Rp, measured in tandem with bronchoalveolar lavage (BAL), appeared unaffected by repetitive O_3 exposure. Atropine was withheld in four subjects prior to bronchoscopy with no apparent effect on the outcome. The respective Rp values in cm H_2O/ml/min (mean, range) after O_3 and Fa were as follows: with atropine pretreatment, 0.027 (0.002 to 0.066) versus 0.027 (0.003 to 0.063); with atropine withheld, 0.014 (0.003 to 0.038) versus 0.023 (0.003 to 0.069).

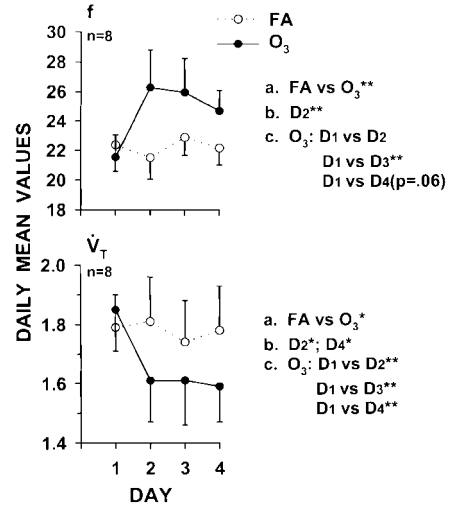

Figure 4. Persistent effect of repeated O_3 exposure on respiratory frequency (f) and tidal volume (V_T) during exercise. Mean, SEM. See legend to Figure 1 for description of components of the statistical model. A more rapid, shallow pattern of breathing was established by Day 2. Each point represents the mean of all six measurements made that day. *$p < 0.05$; **$p < 0.01$; FA = filtered air; O_3 = ozone; D = day; BPM = breaths/min; L = liters.

TABLE 3. BALF CONTENTS, DAY 5,* FOLLOWING REPETITIVE EXPOSURES TO FA AND O_3

	FA	O_3	p† Value
Cell counts			
Fluid recovery, ml	54.2 ± 3.8	52.9 ± 3.8	0.80
Total cells, $\times 10^6$	11.6 ± 1.5	14.7 ± 1.4	0.26
Macrophages, %	86.3 ± 2.2	84.5 ± 2.2	0.40
Lymphocytes, %	12.0 ± 2.1	13.2 ± 2.1	0.48
Neutrophils, %	1.0 ± 0.4	1.8 ± 0.2	0.12
Neutrophils, $\times 10^4$	9.8 ± 2.8	25.6 ± 3.6	0.04
Eosinophils, %	0.1 ± 0.05	0.1 ± 0.04	0.74
Epithelial cells, %	0.5 ± 0.2	0.4 ± 0.1	0.79
Biochemistry			
Albumin, μg/ml	40.7 ± 9.1	44.5 ± 9.3	0.78
Fibrinogen, ng/ml	74.5 ± 27.1	91.9 ± 22.7	0.78
Kinins, pg/ml	164.5 ± 26.7	433.9 ± 206.4	0.09

Definition of abbreviations: BALF = bronchoalveolar lavage fluid; FA = filtered air; O_3 = ozone.

* Mean ± SE; n = 8.

† Wilcoxon signed rank test, FA versus O_3.

DISCUSSION

Functional Response

Repetitive exposure to 0.25 ppm O_3 over a 4-d period elicited two patterns of response, adaptive and persistent. All spirometric and ventilatory variables underwent adaptive changes. The changes varied in day of onset. The SAW_{grp}, a composite of (isoV) $FEF_{25–75}$, $\dot{V}max50$, and $\dot{V}max75$, was first to adapt (Day 1), followed 1 d later by FVC, FEV_1, and by f, and V_T on the last day of exposure (Figures 1 and 3).

Adaptive responses to O_3 involving FVC, FEV_1, symptoms of respiratory irritation, bronchial reactivity, exercise performance, and, more recently, a number of inflammatory markers are well documented (1, 4–6). Functional adaptation, as measured by FEV_1, has been seen despite cellular and biochemical evidence in BALF of ongoing inflammation, and of neutrophil infiltration in bronchial mucosa (6). To our knowledge, volume-corrected spirometric measures or other indices of small airway function have not been examined before in a similar way. Evidence of an adaptive change in breathing pattern with repetitive O_3 exposure, particularly of f, was first described by Foxcroft and Adams (19, Figure 2). (That acute exposure elicits progressive tachypnea during exercise at concentrations at or above 0.20 to 0.25 ppm is also well documented [1, Table 7-1].) The onset of an adaptive response appears to be influenced by both the level of exposure and sensitivity of the individual to O_3. More intense exposure and increased sensitivity are likely to delay the onset (1, 20–22).

The small airway composite measure alone among spirometric variables displayed a persistent preexposure baseline depression, beginning with Day 2 (Figure 2). This dichotomy between the SAW_{grp} and both FVC and FEV_1 accords with earlier results seen following acute O_3 exposure, namely, that FVC and FEV_1 recovered more rapidly than (isoV) $\dot{V}max$ (9, 10). Persistent depression of the FVC and FEV_1 baselines have generally been uncommon or marginal during repetitive O_3 exposure at concentrations ⩽ 0.4 ppm (2, 3, 5, 20, 22).

Our method of adjusting the three components of the SAW_{grp} for O_3-induced changes in FVC (thereby solving for intrinsic changes in small airway caliber) relies on two assumptions, namely, that O_3 exposure has little or no effect on either residual volume (RV) or lung elastic recoil. To our knowledge, neither assumption has been tested experimentally with repetitive exposure. Hazucha and coworkers (23) concluded that the reduction in FVC following acute O_3 exposure (0.5 ppm × 2 h) was not attributable to the small increase in RV (+11%, $p < 0.05$) or the tendency toward an increase in elastic recoiling force they observed. Beckett and coworkers (0.4 ppm × 2 h) (24) and our group (0.35 ppm × 130 min) (8, 9) found no significant change in RV following acute exposure. It is to be noted that an increase in RV, as might occur if partially obstructed small airways closed prematurely during forced expiration, would act to diminish the calculated reduction in isovolumetric flow. Similarly, an increase in elastic recoil would, if transmitted to the outer walls of intrapulmonary airways, act as a distending force to preserve maximal flow at low lung volumes (25).

Although we did not determine the time required for the small airway dysfunction to remit, we suspect it is tied to complete remission of the inflammatory process, as discussed in the next section. Whether the dysfunction may serve as a forerunner of more permanent loss is an open question. Permanent loss might follow in the event that O_3 exposure is repeated over extended periods of time or individuals are unusually reactive to O_3, as Subjects 2 and 4 appeared to be. Recently, Künzli and coworkers (26) reported finding a significant correlation between estimated lifetime exposure to ambient O_3 among college freshmen (lifetime residents of California) and impaired small airway function. The latter was assessed spirometrically, based on $FEF_{25–75\%}$ and $FEF_{75\%}$; FVC and FEV_1 were unaffected. Additional epidemiological and field studies to test possible intermediate and long-term effects of photochemical air pollution on small airways appear indicated.

Inflammatory Response

In our study of the acute effects of O_3, the levels of albumin, fibrinogen, kinins, and PMN (both absolute number and percentage of total cell count) were significantly elevated 24 h postexposure (10). In the present study, only the absolute number of PMN remained elevated by Day 5. In the earlier study, the changes in fibrinogen, a marker of plasma transudation, and (isoV) $FEV_{25–75}$ were inversely and strongly correlated ($r = -0.88$; $p = 0.001$; $n = 8$); the spirometric measurement had also been made 1 d postexposure and, in effect, represented a "persistent" change of at least 24 h. In the present study, the changes in fibrinogen correlated inversely but weakly with the degree to which the small airway baseline, expressed as $SAW_{factor1}$, was depressed ($r = -0.46$; $p = 0.26$; $n = 8$). Together, these findings are consistent with a general waning of the exudative component of the inflammatory response following repetitive O_3 exposure, as reported by other investigators (4–6). The exceptionally high levels of exudative markers in two subjects (Numbers 2 and 4) also conform to previous evidence of wide differences in inflammatory activity among healthy subjects following repetitive exposure (5, Figure 3).

Mechanisms

We propose that both the adaptive and persistent functional responses were byproducts of inflammatory changes set in motion on the first day of exposure. The former reflects the protective action of inflammation and the latter reflects the functional inefficiencies—or costs—associated with that protection. We use the term "inflammation" broadly to include increased vascular permeability, cellular influx, release of mediators, and any responses these primary events may entrain.

Although the acute inflammatory response is emblematic of tissue injury, it also is viewed as an adjustment that helps confine injury and defends against further environmental challenge (27). Plasma exudate contains elements that can inter-

rupt the cascade of chemical reactions associated with lipid peroxidation (28–30), scavenge reactive oxygen species (albumin, a traditional marker of transudation, is, itself, an effective antioxidant [29]), limit tissue injury, and stimulate repair and regeneration (31). Mucorrhea and increased airway smooth muscle tone, frequent accompaniments of inflammation, may interfere mechanically with local gas flow and additional O_3 transport to inflamed sites. That the epithelial lining fluid, as a result of inflammatory changes in volume and composition, is likely to be a more effective barrier against oxidative stress is implicit in the O_3 transport model (32, Figures 2 and 4). Several of these factors have indeed been cited as promoters of functional and symptomatic adaptation to O_3.

Admittedly, the generally weak correlations between inflammatory and persistent functional changes provide little support for our proposition that the latter reflect the inefficiencies of an otherwise protective response. Nonetheless, we do not regard them as voiding the proposition. Although useful diagnostically, bronchoalveolar lavage is an invasive procedure that cannot provide an adequate accounting of a process as complex and nonlinear in its dynamics as inflammation. Estimates of the interdependencies between inflammatory and functional responses are therefore subject to considerable uncertainty. Insofar as functional and inflammatory responses follow different time courses, the apparent strength of their interdependency will be sensitive to the timing of each measurement.

Tachypnea

Stimulation of afferent vagal fibers (bronchial C-fibers) is considered the chief basis for the rapid, shallow breathing (during exercise), chest discomfort, and reduced inspiratory capacity associated with acute O_3 exposure (23, 33, 34). It is therefore difficult to reconcile the persistent tachypnea seen during repetitive exposure with the absence of analogous changes in FVC and FEV_1, unless another mechanism keyed solely to the control of breathing is invoked. A plausible candidate for this role is a change in the viscoelastic properties of the distal lung, triggered by the tissue injury and inflammation that accompany the first day's exposure to O_3. The rationale for this potential mechanism was developed decades ago to explain breathlessness during exercise.

The pattern of breathing used to achieve a specific $\dot{V}_E$ is highly variable (35). Campbell (36) postulated that information about respiratory muscular and mechanical behavior is processed unconsciously by the cortex, which exerts "unconscious control" of the breathing pattern. If, as a consequence of O_3-induced injury and inflammation, tissue viscance (Vti) were increased out of proportion to any narrowing of the large conducting airways, more rapid, shallow breathing would likely follow, especially with exercise (37, 38). This adjustment in breathing pattern should mitigate respiratory discomfort or breathlessness arising from the altered mechanical behavior of the distal lung.

The premise about the O_3-induced change in Vti is untested. Certainly, constriction of large, central airways, as reflected in an increase in airway resistance (SRaw or Raw), is not a prominent feature of the response to acute exposure (17, 23, 34) and should not exact a high cost in work during tachypnea. Moreover, with repetitive exposure, SRaw adapts readily and has shown little or no baseline elevation (5, 39). In our study, the underlying adjustment of the respiratory pattern did not occur until Day 2 when, presumably, tissue injury and inflammatory changes were more fully established. The proposed change in Vti does not imply a parallel change in Rp, which was unaffected. The two measures may be governed by anatomically distinct contractile elements, as evidenced by their dissimilar responses to histamine and leukotriene C_4 aerosols (13).

Peripheral Airway Resistance

As noted above, Rp was unaffected by repetitive O_3 exposure notwithstanding the evidence of persistent small airway narrowing and inflammation of the peripheral lung. This dichotomy between collateral and small airway responses was also seen after acute O_3 exposure (10). Following the acute exposure, all subjects prior to bronchoscopy received atropine, a cholinergic antagonist, and lidocaine, a topical anesthetic. In the present study, atropine was withheld from half of the subjects without affecting the outcome. It would appear that either the dose of O_3 to the airways responsible for Rp was negligible relative to the dose to small airways, the former airways were highly resistant to O_3, or that lidocaine premedication abolished any increase in Rp that might have occurred. We favor the first of these possibilities. Consistent with our findings is the recent observation that in anesthetized, mechanically ventilated dogs, 0.2 ppm O_3 administered for 6 h by endotracheal tube had no effect on Rp unless the animals were pretreated with probenecid, an inhibitor of endogenous antioxidant transport. In contrast, pulmonary resistance increased in both untreated and pretreated animals (40).

Collateral channels having the structural characteristics of respiratory bronchioles and alveolar ducts appear to be chiefly responsible for Rp (12). They represent diffusion pathways in normal lungs. Small airways, which dominate maximal expiratory flow at intermediate to low lung volumes, are likely to include subsegmental bronchi and membranous bronchioles. Opposing views have been expressed about whether collateral channels have a significant role in gas exchange in the normal lung (12, 41). Our results are consistent with their having a minor role, especially among healthy young adults.

In summary, we observed two patterns of functional response among healthy, young adults exposed repetitively to O_3, adaptive and persistent. All spirometric variables, as well as f and V_T, underwent adaptive changes. Spirometric evidence of persistent change was confined to small airways; rapid, shallow breathing during exercise also persisted. Neutrophilia in BALF was evident 1 d following the end of O_3 exposure. We suggest that both types of functional response are linked causally to inflammation. The adaptive component is attributable at least in part to a reduction in local tissue dose during repetitive exposure that is likely to result from the biochemical, mechanical, and morphological changes set in motion by inflammation. The persistent component represents the inefficiencies incurred through inflammation. Whether the persistent small airway dysfunction is a forerunner of more permanent change in the event that oxidant stress is extended over lengthy periods of time is unclear.

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APPENDIX

We knew from experience gained in this and other laboratories of the large coefficient of variation associated with $\dot{V}$max at low lung volumes (11, 16) where, theoretically, $\dot{V}$max is most reflective of small airway caliber (25). We judged that eight subjects would suffice for an adequate test of our hypothesis about the asymmetries between volume-dependent (FEV_1, FVC) and isovolumetric small airway ($FEF_{25–75}$, $\dot{V}$max50, $\dot{V}$max75) measures in response to repetitive O_3 exposure—if the variability of these data could be reduced. The principal components statistical model served this end. The model is intended to reduce the number of measures that are related conceptually by grouping them, thereby reducing the variability of the same data. A table of the analytic results affirming the appropriateness of grouping the three measures into one functional entity (SAW_{grp}) is available upon request. The adaptive and persistent responses among the individual components are shown in Figures 5 and 6, respectively. Their marked variability relative to that of SAW_{grp} (Figures 1 and 2) is evident. The greater clarity of outcome associated with the SAW_{grp} analysis is also evident. We believe the model holds promise as a means of tracking small airway function. But because the observation of persistent small airway narrowing attending repetitive O_3 exposure is novel and is based on a limited number of test subjects, replication is needed.

Each individual SAW_{grp} value retained the original number of observations (n = 3). To permit correlations between SAW_{grp} and other variables, that is, BALF contents, f and V_T, it was necessary to reduce n from three to one per subject. This step involved normalizing and then recombining the three spirometric

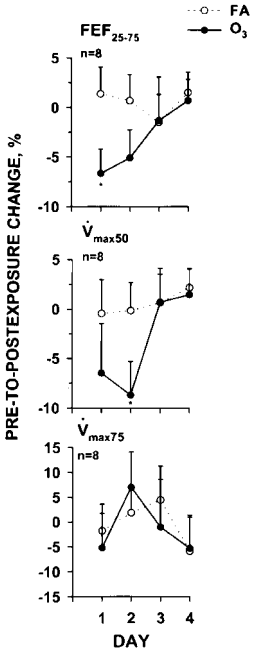

Figure 5. Adaptive changes among individual isovolumetric components of SAW_{grp}. The standard errors for component d (within day differences) of the "mixed effects" analysis are shown. *$p < 0.05$; FA = filtered air; O_3 = ozone.

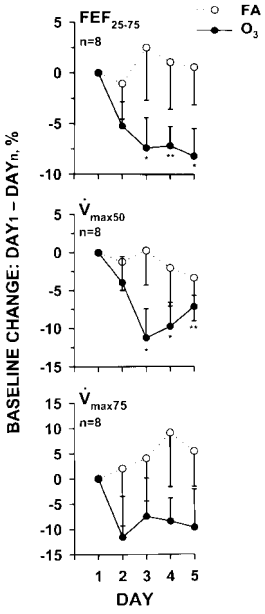

Figure 6. Persistent changes among individual isovolumetric components of SAW_{grp}. The standard errors for component c (day-to-day baseline differences) of the "mixed effects" analysis are shown. *$p < 0.05$, **$p < 0.01$; FA = filtered air; O_3 = ozone. As in Figure 5, V̇max75 showed the largest measurement error.

variables based on their relative "weightings." The weightings were calculated with a second principal components model and were based on the percentage changes in each of the variables associated with exposure to FA and O_3. This modified value is symbolized by $SAW_{factor1}$. To calculate correlation coefficients (r), all data were transformed logarithmically.