

Is There an Association Between Lifetime Cumulative Exposure and Acute Pulmonary Responses to Ozone?

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Objective: To investigate the potential effects of lifetime cumulative ozone (O_3) exposure on acute pulmonary responses to O_3 . **Methods:** Fifteen healthy subjects from a larger cohort of young adults were exposed to 200 ppb O_3 for 4 hours followed by bronchoscopy and bronchoalveolar lavage 18 hours later. Lung function, symptom questionnaires, and blood samples were obtained before and after each exposure. Subjects' lifetime cumulative O_3 exposures were estimated from residential histories and air-quality monitoring data. **Results:** Acute exposure to O_3 caused decrements in forced expiratory volume in 1 second (FEV_1), maximal mid-expiratory flow rate (FEF_{25-75}), and forced expiratory flow rate at 75% of forced vital capacity (FEF_{75}), and an increase in plasma clara cell protein (CC16) level. Changes in CC16 and lower respiratory symptoms, but not in lung function, were positively correlated with lifetime cumulative O_3 exposure. **Conclusion:** Higher lifetime cumulative O_3 exposure was associated with airway injury and respiratory symptom responses, but not with airway inflammatory or lung function responses, to acute O_3 exposure. (J Occup Environ Med. 2008;50:341-349)

Ozone (O_3) is an important component of urban air pollution that causes cellular and tissue injury through generation of oxidative stress.¹⁻⁴ Human studies have demonstrated that short-term exposure to O_3 causes acute changes in lung function as well as cellular and biochemical evidence of airway inflammation and injury.⁵⁻¹⁰ Moreover, human studies have shown that with repeated short-term exposures, while oxidative injury continues to be present, some O_3 -induced physiologic and inflammatory responses undergo attenuation.¹¹⁻¹⁵ In addition, several studies have documented that subjects have attenuated lung function responses to acute controlled O_3 exposure during the summer months in areas that are characterized by high ambient O_3 concentrations.^{16,17} The mechanism of this attenuation is not well understood but may be related to a suppressive or adaptive pathway in the airways.

Human studies also have documented the presence of considerable between-subject variability in both the lung function and the inflammatory responses to O_3 -induced oxidative stress.¹⁸⁻²² Although the between-subject variability of lung responses to O_3 has been well established, the basis of this variability is not well understood. Epidemiologic and controlled exposure studies have suggested various genetic²³⁻²⁵ and environmental factors^{26,27} as the source of the between-subject variability in lung responses to O_3 . In

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particular, this variability may be mediated, at least in part, by the adaptive (or maladaptive) responses of the airways to lifelong O₃ exposure that may include biochemical (eg, increased antioxidant enzyme expression) and structural (eg, airway remodeling) changes.^{28,29} Investigation of the relation between lifelong O₃ exposure and acute lung responses to O₃ may help to understand better the mechanisms of this between-subject variability in lung responses.

On the basis of above observations, we hypothesized that individuals with a high level of lifelong exposure to O₃ would have different (attenuated) acute responses to controlled O₃ exposure. Our research question was whether there is a relation between lifetime cumulative exposure to O₃ and acute responses to controlled O₃ exposure. To answer this question, we studied 15 subjects from our cohort of young, healthy Californian adults³⁰ and analyzed their acute lung function and airway injury/inflammatory responses to O₃ in relation to their estimated lifetime cumulative O₃ exposure.

Materials and Methods

Subjects

We recruited 15 subjects from a cohort of young, healthy students of University of California (UC) Berkeley who participated in our study of the association between chronic exposure to O₃ and lung function³⁰ to undergo acute O₃ exposure. Briefly, the UC Berkeley cohort was a convenience sample of students who were entering their first undergraduate year at UC Berkeley between the years 2000 and 2002. The subjects were eligible for the overall study if they met the following criteria: 1) lifelong resident of Los Angeles or San Francisco areas; 2) lifetime never smoker, 3) no history of chronic respiratory disease; and 4) no physical impairment that would hinder performance of spirometry. The cohort consisted of 255 students

(58% women; mean \pm SD age of 18.5 \pm 0.6).

The subjects from the UC Berkeley cohort were eligible for the acute O₃ exposure study if their maximum mid-expiratory flow (FEF₂₅₋₇₅) was in either the upper or lower 10th percentile of FEF₂₅₋₇₅ distribution based on the combined data from our two studies of UC Berkeley freshmen.^{30,31} We used these criteria to compare subjects with possible O₃-induced small airway remodeling to those without remodeling. Primate models of cyclical, chronic exposures to O₃ have established clearly that airway remodeling occurs at the level of the respiratory bronchiole as a consequence of these exposures.³² Pathologic correlations with measures of lung function in humans³³ have documented that FEF₂₅₋₇₅ is a measure that may reflect the function of small airways (<2 mm in diameter). We have shown in several previous articles that the most consistent associations with lifetime cumulative exposure to O₃ are with FEF₂₅₋₇₅.^{30,31} Fifteen subjects were recruited for the acute O₃ exposure study and 9 of the 15 subjects underwent bronchoscopy after O₃ exposure.

The subjects denied any history of cardiac or pulmonary diseases or any respiratory infections within 6 weeks before the onset of O₃ exposure and did not use any supplemental vitamin C or E during the period of the study. All of the subjects received financial compensation for their participation. The Committee for the Protection of Human Subjects at University of California, Berkeley and the Committee on Human Research at the University of California, San Francisco approved all procedures.

Lifetime Cumulative Ozone Exposure Estimates

Lifetime cumulative O₃ exposure for each subject in this study has been reported previously.^{30,31} Briefly, lifetime residential history was reconstructed with a standard-

ized questionnaire and O₃ concentrations were assigned for each month of life to each residential location. Air-quality data were acquired from the California Air Resources Board (ARB, CD No. PTSD-02-017-CD), the Aerometric Information Retrieval System (AIRS), and from special requests to ARB. Monthly mean measures of O₃ were interpolated spatially from air-quality monitoring stations to the residence locations with inverse distance weighting and a maximum of three monitoring stations for each interpolation (maximum interpolation radius of 50 km; most monitors less than 8 km). The details and reliability of the exposure assignment method have been published.^{31,34} Briefly, we fit two basic models to estimate lifetime pollutant O₃ exposure. There was no significant difference in the association between lifetime O₃ exposure and lung function between the two models. In this article, we used the so-called "ecological" model, which omitted estimates of time spent outdoors and used only the residence-specific monthly average, interpolated pollutant concentrations.

Lung Function Measurements

Spirometry was performed on a dry rolling-seal spirometer (Collins Survey; Warren E. Collins, Co Braintree, MA). Mean values for forced vital capacity (FVC), forced expiratory volume in 1 second (FEV₁), FEF₂₅₋₇₅, and forced expiratory flow at 75% of FVC (FEF₇₅) were calculated from three acceptable FVC maneuvers⁴ obtained approximately 30 seconds apart.

Exposure Chamber and Atmospheric Monitoring

All exposures took place in a chamber ventilated with filtered air at 20°C and 50% relative humidity to which O₃ was added. The stainless steel and glass chamber, 2.5 \times 2.5 \times 2.4 m (Model W00327-3R; Nor-Lake, Hudson, WI), was custom-built and designed to maintain chamber temperature and relative humidity within 2.0°C and 4%, respectively,

of the set points (DSC 8500; Johnson Controls, Poteau, OK).³⁵ Relative humidity and temperature were recorded every 30 seconds and averaged over each exposure.

Ozone was produced using a corona-discharge O₃ generator (Model T408; Polymetrics, Inc, San Jose, CA) and its concentration was monitored with an ultraviolet light photometer (Model 1004AH; Dasibi, Glendale, CA). Ozone concentration was measured every 30 seconds, displayed in real-time (LabView 2; National Instruments, Austin, TX), and stored by a computer (Model Iisi; Apple Computer Inc, Cupertino, CA). The O₃ analyzer was calibrated periodically with an O₃ transfer standard (Model 1003PC; Dasibi, Glendale, CA) by the California Air Resources Board.

Ozone Exposure Protocol

The experimental protocol involved exposure to 200 ppb O₃ for 4 hours. The subjects exercised for first 30 minutes of each hour and then rested for the remaining 30 minutes of the hour. The exercise consisted of either walking or running on a treadmill (Model No. M9.1; Precor, Bothel, WA) or pedaling a cycle-ergometer (Model No. 90818e; Monark, Varberg, Sweden). The exercise intensity was adjusted for each subject to achieve a target V_E of 25 L/min/m² body surface area. During exercise, V_E was calculated from tidal volume and breathing frequency measured with a pneumotachograph (Model No. 3; Fleisch, Rudolph Instruments, Kansas City, MO) at the 10-minute and 20-minute intervals of each 30-minute exercise period. Subjects remained inside the chamber for the entire 4-hour exposure period.

Baseline spirometry for each subject was measured immediately before and after each exposure. A blood specimen was collected immediately before (baseline) and 18 hours after each O₃ exposure. Blood samples were obtained at 18-hours postexposure, because the subjects

required the insertion of an intravenous catheter before bronchoscopy. In addition, blood samples were obtained from five subjects immediately after O₃ exposure. Blood was collected by venipuncture in tubes that contained heparin and was processed to separate plasma and other fractions as previously described.³⁶ Plasma samples were stored at -80°C according to best repository requirements (ISBER 2005).

Respiratory Symptoms

Self-administered symptom questionnaires were completed immediately before and after each exposure as well as before bronchoscopy. Symptom questionnaires consist of a 5-point rating scale (0 = none and 4 = severe) for lower respiratory (chest discomfort or tightness, chest pain on deep inspiration, shortness of breath, cough, phlegm or sputum production, and wheezing), upper respiratory (throat irritation and nasal irritation), and nonrespiratory (anxiety, eye irritation, headache, and nausea) symptoms. To compare the change across exposure, the symptom scores within lower respiratory, upper respiratory, and nonrespiratory categories were averaged to obtain a single score for each category.

Bronchoscopy and Lavage Procedures

Bronchoscopies were performed 18 ± 2 hours after the exposure, because previous studies by both our laboratory and other investigators have documented the presence of an O₃-induced inflammatory response in many subjects at this time point.^{6,8,37} Our laboratory's procedures of bronchoscopy and BAL have been discussed in detail previously.^{8,37} Briefly, after intravenous access, upper airway topical anesthesia, and moderate sedation with intravenous midazolam and fentanyl were established, the bronchoscope (FB 18x; Pentax Precision Instruments Corp, Orangeburg, NY) was introduced through the mouth and vocal cords into the airways. The

bronchoscope was then directed into the right middle lobe where lavage was performed with two 50-mL aliquots of 0.9% saline warmed to 37°C. The first 15 mL of fluid returned from the first 50-mL aliquot was collected separately and labeled bronchial fraction (BFx), whereas the remaining fluid returned was labeled BAL. Both lavage samples were placed immediately on ice. After bronchoscopy, each subject was observed for an approximate 2-hour recovery period.

Total cells were counted on uncentrifuged aliquots of BFx and BAL with a hemocytometer. Differential cell counts were obtained from slides prepared with a cytocentrifuge (Cytospin 2; Shandon Southern Products, Ltd, Astmoor, UK), 25× g for 5 minutes, and stained with Diff-Quik (American Scientific Products, McGaw Park, IL), as previously described.⁸ BFx and BAL fluids were then centrifuged at 180× g for 15 minutes, and the supernatant was separated and recentrifuged at 1200× g for 15 minutes to remove any cellular debris before freezing at -80°C.

Measurement of Biochemical Constituents of BFx and BAL Supernatants

Biochemical assays were performed on BFx and BAL supernatants that had been frozen at -80°C. Interleukin (IL)-6 and IL-8 were measured with ELISA immunoassays (R&D Systems, Minneapolis, MN) in accordance with the manufacturer's instructions. BAL total protein was determined with the BCA Protein Assay ELISA kit (Pierce, Milwaukee, WI).

Plasma Clara Cell Protein Assay

The plasma was separated from blood cells by centrifugation, diluted 1:5, and analyzed in duplicate. The concentration of Clara cell protein (CC16) was measured by a sandwich enzyme ELISA kit available from BioGene (BioGene, US Biological;

Swampscott, MA) based on the manufacturer's protocol on the Spectra-max Plus (Molecular Devices, Sunnyvale, CA) microplate reader. The variability in readings was less than 3%.

Statistical Analysis

All data were entered into a Microsoft Access 2000 database (Microsoft Corp, Seattle, WA). Data were then analyzed with Stata 8.0 software (Stata Corporation, College Station, TX).

If the measured variable had a normal distribution, the *t* test was used to compare pre-exposure and postexposure values; otherwise, Wilcoxon signed rank test was used. Statistical correlations between estimated monthly average lifetime O₃ exposure and outcomes of controlled O₃ exposure were evaluated by Spearman's rank correlation. A *P* value of 0.05 was considered statistically significant in all data analyses. All correlation analyses for plasma CC16 levels were done using the 18-hours postexposure values.

During graphical analysis of the correlation between lifetime cumula-

tive O₃ exposures and changes in plasma CC16 levels, we noted a potential influential outlier point (one subject with lowest lifetime cumulative O₃ exposure and highest change in CC16). Removal of this point improved the correlation from *R* = 0.31 (*P* = 0.29) to *R* = 0.63 (*P* = 0.02). On the basis of this observation, we elected to exclude the CC16 value for this subject from the correlation analysis. The graph with the influential point included is available in the on-line supplement (Fig. C).

Results

Subjects Characteristics

Characteristics of the individual subjects and their estimated lifetime cumulative O₃ exposures are listed in Table 1. The distribution of estimated average monthly lifetime O₃ exposures in our acute exposure study subgroup was similar to that of the entire UC Berkeley student cohort (median [interquartile range]: 29.7 [25.0, 38.1] ppb vs 33.5 [27.4, 44.5] ppb, respectively).

Exposure Data

Ozone concentration during controlled exposures was (mean ± SD) 206 ± 8 ppb. The exposure chamber temperature and humidity were 19.4° ± 0.5°C and 56.1% ± 2.7%, respectively. The subjects' average exercise V_E was 38.5 ± 6.4 L/min (22.5 ± 2.4 L/min/m² body surface area).

Lung Function

The pre- and postexposure values for, and the changes across exposures in, FVC, FEV₁, FEF₂₅₋₇₅, and FEF₇₅ are shown in Table 2. As expected, O₃ exposure caused a significant decrease in all lung function indices across exposure. These acute O₃-induced lung function changes did not correlate with subjects' estimated lifetime cumulative O₃ exposure (*R*_{FEV₁} = 0.08, *P* = 0.79; *R*_{FEF₂₅₋₇₅} = -0.02, *P* = 0.95; *R*_{FEF₇₅} = -0.27, *P* = 0.34) (Fig. 1 and Figs. A and B in on-line supplement). In addition, there was no correlation between acute O₃-induced lung function changes and an index of intrinsic airway size (the pre-exposure

TABLE 1
Subject Characteristics

Subject No.	Sex	Age (yr)	FEV ₁		FEF ₂₅₋₇₅		FEF ₇₅		Average Lifetime O ₃ (ppb)
			FEV ₁ (L)	(% Predicted)*	FEF ₂₅₋₇₅ (L/s)	(% Predicted)*	FEF ₇₅ (L/s)	(% Predicted)*	
1	F	19	2.99	93.0	2.60	59.0	1.28	38.7	22
2	M	20	3.66	95.0	4.41	93.3	1.97	66.0	23
3	M	20	2.58	82.0	2.06	50.3	1.02	37.3	25
4	M	21	3.37	80.9	3.06	65.0	n/a	n/a	25
5	M	21	4.74	100.0	5.29	105.5	3.41	91.0	27
6	F	20	2.22	108.0	1.92	57.0	0.97	35.3	29
7	F	20	3.21	104.0	4.88	118.7	2.49	90.3	29
8	F	20	3.95	102.8	4.48	106.3	2.94	92.0	30
9	F	18	3.41	109.0	5.67	132.0	4.05	127.0	30
10	F	19	3.43	103.0	5.37	118.3	2.90	87.0	31
11	M	18	5.21	125.0	8.45	164.0	5.43	168.0	34
12	F	20	2.19	72.0	2.39	65.1	1.41	47.0	38
13	M	18	3.79	98.0	3.22	66.3	1.85	60.3	43
14	F	20	2.89	83.0	2.72	61.7	1.74	63.0	44
15	F	20	3.39	107.0	3.30	80.3	1.73	62.7	56
Mean		19.6	3.40	96.6	4.00	89.5	2.37	76.12	32.3
SD		1.0	0.83	11.4	1.8	33.5	1.3	37.2	9.3

Mean values of the three trials of spirometry performed immediately prior to exposure to ozone are shown.

FEV₁ indicates forced expiratory volume in 1 s; FEF₂₅₋₇₅, maximal mid-expiratory flow rate; FEF₇₅, forced expiratory flow rate at 75% of forced vital capacity; average lifetime O₃, estimated monthly average O₃ concentration over the lifetime of the subject in parts per billion (ppb) (30); M, male; F, female; n/a, not available.

*Crapo et al.⁵²

TABLE 2
Spirometry Indices Before and After Ozone Exposure (N = 15)

Spirometry Indices	Pre-Exposure	Postexposure	Change	P Value
FVC (L)	3.8 [3.4, 4.4]	3.4 [2.9, 3.9]	-0.5 [-0.2, -0.6]	<0.001
FEV ₁ (L)	3.4 [2.9, 3.8]	2.8 [2.6, 3.1]	-0.4 [-0.3, -0.7]	<0.001
FEF ₂₅₋₇₅ (L/s)	3.3 [2.6, 5.3]	2.7 [2.1, 4.4]	-0.5 [-0.2, -1.2]	<0.001
FEF ₇₅ (L/s)	1.9 [1.4, 2.9]	1.5 [1.3, 2.8]	-0.3 [-0.1, -0.4]	0.006

Values shown are median [interquartile range].

P values are for paired Wilcoxon signed rank test between pre- and postexposure measurements.

FVC indicates forced vital capacity; FEV₁, forced expiratory volume in 1 s; FEF₂₅₋₇₅, maximal mid-expiratory flow rate; FEF₇₅, forced expiratory flow after 75% of expelled volume.

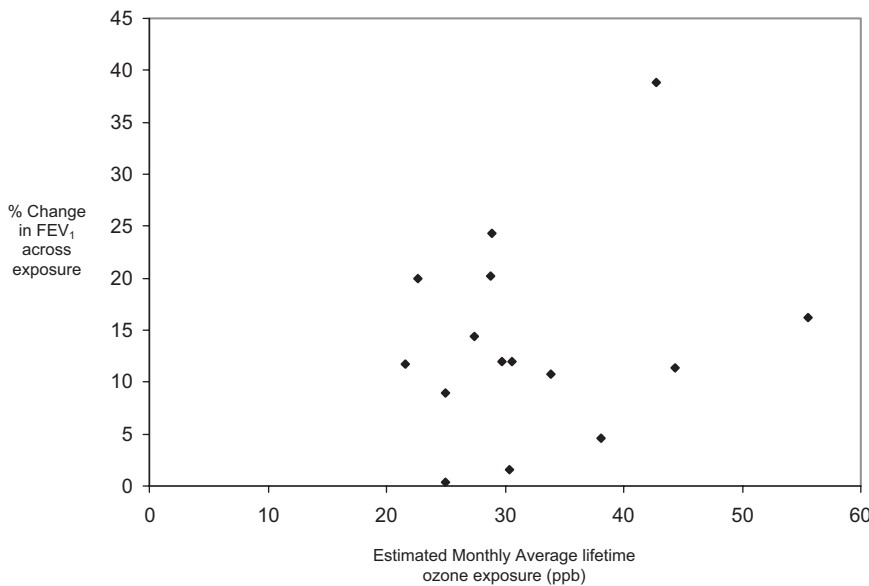


Fig. 1. Correlation between average lifetime ozone exposure and percent change in FEV₁ across acute ozone exposure (N = 15; Spearman R = 0.24; P = 0.39).

FEF₂₅₋₇₅/FVC) that we have previously shown to be a determinant of the relationship between lifetime cumulative O₃ exposure and lung function.³⁰

Symptoms

The subjects reported a worsening of their lower respiratory symptoms after exposure to O₃ (median [interquartile range]: 1.15 [0.7, 1.3]; Wilcoxon signed ranks P = 0.001). In addition, the subjects reported a slight worsening of their upper respiratory (throat irritation and nasal irritation) symptoms across O₃ exposures (0.25 [0.0, 1.5]; Wilcoxon signed ranks P = 0.01), but there was no significant change in the nonrespiratory (anxiety, eye irritation, headache, and nausea) symptoms (0.0 [0.0, 0.5]; Wilcoxon signed ranks P = 0.21).

The change in subjects' lower respiratory symptoms and the estimated lifetime cumulative O₃ exposure had a positive correlation (R_{lower resp} = 0.53, P = 0.05) (Fig. 2). Upper respiratory and nonrespiratory symptoms did not correlate with estimated lifetime cumulative O₃ exposure.

Plasma Clara Cell Protein

The plasma level of CC16, a marker of epithelial cell injury, increased by (median [interquartile range]) 1.8 [-0.2, 3.8] ng/mL (n = 14; P = 0.01) from a pre-exposure baseline level of 7.0 [4.9, 8.1] ng/mL to 8.0 [6.1, 10.0] ng/mL 18 hours after O₃ exposure. Exclusion of the outlier point only minimally affected this results (1.6 [-0.2, 3.4] ng/mL;

n = 13; P = 0.02). The immediate postexposure plasma level of CC16 was only measured in five subjects and showed a significant increase of 6.1 [4.1, 7.9] ng/mL (n = 5; P = 0.04) from a baseline level of 7.4 [7.3, 8.3] ng/mL to 13.4 [13.2, 13.6] ng/mL immediately postexposure before decreasing to 10.2 [8.0, 13.1] ng/mL 18 hours after O₃ exposure. The acute change in CC16 plasma levels was positively correlated with subjects' estimated lifetime cumulative O₃ exposure (R_{CC16} = 0.63, P = 0.02) (Fig. 3). The changes in plasma CC16 levels and lower respiratory symptoms showed a positive but not significant correlation (R = 0.36, P = 0.22).

Lavage Cell Counts

The total number of leukocytes and the percentage of neutrophils in BFX and BAL fluid were elevated as expected after O₃ exposure (Table A in on-line supplement). The lavage neutrophilia did not correlate with estimated lifetime cumulative O₃ exposure. There were no significant correlations between BFX or BAL fluid total leukocyte or neutrophil counts and changes in either lower respiratory symptoms or plasma CC16 levels.

Lavage Total Protein and Cytokines

The concentrations of IL-6 and IL-8 cytokines and total protein in BFX and BAL are shown in Table B in on-line supplement. There were no significant correlations between the concentrations of total protein or the above-mentioned cytokines and the subjects' estimated lifetime cumulative O₃ exposure. There were no significant correlations between lavage total protein, IL-6, or IL-8 and changes in either lower respiratory symptoms or plasma CC16 levels.

Variability in Pulmonary Responses

The subjects showed considerable variability in their physiologic (FEV₁, FEF₂₅₋₇₅, and FEF₇₅) and

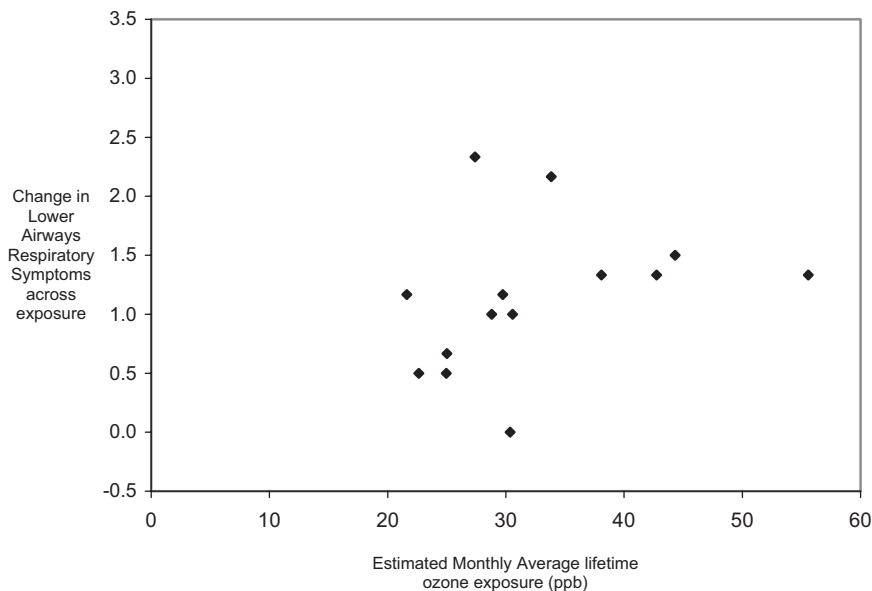


Fig. 2. Correlation between average lifetime ozone exposure and change in lower respiratory tract symptoms across acute ozone exposure ($N = 15$; Spearman $R = 0.53$; $P = 0.049$).

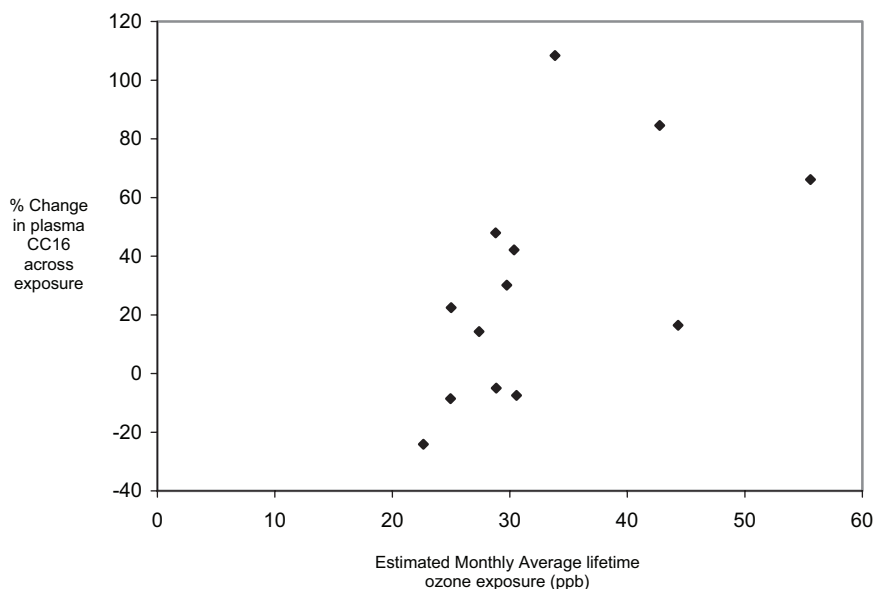


Fig. 3. Correlation between monthly average lifetime ozone exposure and percent change in plasma CC16 (ng/mL) across acute ozone exposure ($N = 13$; Spearman $R = 0.63$; $P = 0.02$).

inflammatory (CC16) responses to acute O_3 exposure as shown in Table 3. Furthermore, the changes in lung function did not correlate with either changes in plasma CC16, changes in lower respiratory symptoms, or with inflammatory indices in the lavage fluid.

Discussion

Contrary to our hypothesis, in this small sample of young, healthy

adults, we found that higher lifetime cumulative O_3 exposures were associated with greater subjective report of lower respiratory symptoms and larger increase in plasma CC16 levels after acute O_3 exposure. These findings suggest that subjects with higher lifetime cumulative O_3 exposure may be more susceptible and have more severe airway injury in response to acute O_3 -induced oxidative stress. In addition, we found a

significant increase in plasma CC16 level after acute O_3 exposure. Although plasma CC16 has been previously reported to increase within 6 hours after short-term O_3 exposure⁹, our data shows that the epithelial cell injury due to O_3 exposure continues to be present even 18 hours after the end of the exposure.

Despite evidence suggestive of increased susceptibility to acute O_3 -induced airway injury, we did not find any significant correlation between lifetime cumulative O_3 exposure and acute lung function changes after O_3 exposures. This lack of correlation may be at least in part due to different mechanisms responsible for airway injury/inflammation and for lung function changes after acute O_3 exposure.^{38,39} The studies that have examined the relationship between acute O_3 -induced changes in FEV₁ and FVC and subsequent airway inflammation (up to 18 hours following exposure) have shown either no correlation or a negative one^{5,8} although one study did show a significant correlation between acute increases in airway resistance (S_{Raw}) and subsequent airway inflammation.⁴⁰ More recently, a study by Weinmann et al in a small number of subjects showed that decrements in FEF₂₅₋₇₅ measured isovolumetrically persist longer than other O_3 -induced acute decrements in lung function and suggested that these decrements correlate better with the acute O_3 -induced inflammatory response than changes in FEV₁, FVC, and S_{Raw} .⁴¹ Furthermore, other studies have suggested that the mechanism by which O_3 induces decrements in FEV₁ and FVC appears to be due to the involuntary inhibition of inspiratory effort involving C-fibers innervations of airways.^{42,43} The mechanism by which O_3 causes mild increases in S_{Raw} and decreases in FEF₂₅₋₇₅ is less clear, but is presumably in part due to airway narrowing possibly as a result of airway inflammation.^{40,41} Taken together, the data indicate that the mechanisms responsible for airway inflammation or injury

TABLE 3

Percent Change in Spirometry Indices and CC16 of Individual Subjects Across Exposures

Subject No.	Average Lifetime O ₃ (ppb)	FEV ₁ (%)	FEF ₂₅₋₇₅ (%)	FEF ₇₅ (%)	CC16 (%)
1	22	-11.7	-19.6	-16.4	(124.2*)
2	23	-19.9	-27.7	-21.3	-24.1
3	25	-0.4	0.0	-3.9	-8.5
4	25	-8.9	-13.4	n/a	22.5
5	27	-14.3	-10.4	-12.0	14.3
6	29	-24.3	-24.0	-29.9	48.0
7	29	-20.2	-34.8	-42.6	-4.9
8	30	-1.5	-2.5	-4.1	30.1
9	30	-12.0	-4.4	-10.1	42.2
10	31	-12.0	-32.2	-36.9	-7.5
11	34	-10.7	-4.4	7.0	108.4
12	38	-4.6	-7.9	0.0	n/a
13	43	-38.8	-36.3	-27.6	84.5
14	44	-11.4	-2.6	-2.9	16.5
15	56	-16.2	-23.9	-13.9	66.1
Mean	32.3	-13.8	-16.3	-15.3	36.5
SD	9.3	9.6	12.8	14.7	45.3

Values shown are mean \pm SD and are percent change in each value from baseline. A negative value shows a decrease from baseline.

*Outlier point—excluded from analyses; see Statistical Analysis in the Materials and Methods section for details.

Ppb indicates parts per billion; FEV₁, forced expiratory volume in 1 s; FEF₂₅₋₇₅, maximal mid-expiratory flow rate; FEF₇₅, forced expiratory flow after 75% of expelled volume; CC16, plasma clara cell protein, ng/mL, % change at 18-hr postexposure compared with baseline before controlled O₃; n/a, data not available.

and lung function changes after acute O₃ exposure are different, and these distinct mechanisms may be affected in different ways by lifetime cumulative O₃ exposure. Our small sample size does not provide sufficient power to definitely address this issue, but our data do show that CC16, a marker of airway epithelial injury, was correlated with lifetime O₃ exposure whereas lung function changes were not.

Furthermore, we did not find any correlation between lifetime cumulative O₃ exposure and airway lavage neutrophilia, total protein, or IL-6 or IL-8 concentrations in this study. This lack of correlation may be due to our limitation of not being able to perform both pre- and postexposure bronchoscopies. Bronchoscopy on its own can result in significant inflammation, and a pre-exposure bronchoscopy could confound the subsequent postexposure O₃-induced inflammation. Consequently, we only performed postexposure bronchoscopy and used

postexposure inflammatory indices, instead of change in these indices across the exposure, to investigate the relation of lifetime cumulative O₃ exposure to bronchoscopic indices of airway inflammation.

The association between lifetime O₃ exposure and greater increase in respiratory symptoms across acute O₃ exposure that we observed in this study may be multifactorial in nature. Besides airway inflammation and injury, smaller baseline size of airways in those subjects with higher lifetime O₃ exposure may contribute to greater increase in symptoms. Cross-sectional studies of college students at the UC Berkeley and Yale University have compared the lung function of subjects between areas of contrasting O₃ profiles. These studies have shown an association between lifetime exposure to ambient O₃ and decreased FEF₂₅₋₇₅ and FEF₇₅ of healthy adults.^{30,31,44} Another longitudinal study that fol-

lowed school children in Austria for 3 years found exposure to O₃ to be associated negatively with lung function growth.⁴⁵ In contrast, a study of children (10 to 18 years) from 12 Southern California communities did not find an association between lung growth and annual average community-specific O₃ concentrations, although the range of average concentrations across the communities was relatively small (factor of <2.5).⁴⁶ Although more data on the effect of lifelong O₃ exposure on lung growth and airway remodeling are clearly needed, the association between lower respiratory symptoms and lifetime O₃ exposure that we observed in this study may be in part due to reduced lung growth.

Various genetic and environmental factors have been implicated as the cause of between-subject variability that is observed in lung responses to O₃-induced oxidative injury. Epidemiologic evidence has increasingly supported a role for genetic variations (eg, sex, intrinsic airway size, and antioxidant enzymes) as important determinants of susceptibility to O₃-induced oxidative injury.^{23,25,30,47-49} Similarly, environmental factors (eg, smoking and diet) have also been implicated in the variability observed in lung responses to O₃.^{26,27,50,51} In the present study, we found evidence suggesting that the variability in inflammatory/injury responses to acute O₃ exposure may be associated with the subjects' lifetime cumulative exposures. Further studies are needed to investigate the underlying mechanisms of these effects but changes in antioxidant defenses may be one possible explanation.

Although this study had a unique hybrid design in which subjects were recruited for controlled exposure from a larger cohort with well-characterized lifetime O₃ exposure and lung function, it was limited by a small sample size of 15 subjects. In consideration of the complex biology of oxidative injury and the interplay of various ge-

netic and environmental factors, this sample size may not be adequate to demonstrate the effects of subjects' lifetime O₃ exposure on their acute responses to short-term O₃ exposure with precision. A larger study with sufficient power will be needed to resolve the role of various environmental factors and potential interactions in modulating acute responses to O₃-induced oxidative injury.

In conclusion, contrary to our a priori hypothesis, lifetime cumulative O₃ exposure was not associated with attenuation of acute lung function to controlled exposure to O₃ in a subset of young, healthy adults from California. We did observe, however, a significant association between lifetime O₃ exposure and both acute O₃-induced respiratory symptoms and evidence of airway injury. These observations should be confirmed in a future study with a larger sample size.

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