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FINAL REPORT
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Evaluation of Chronic Obstructive Pulmonary Disease Patients for Ozone Sensitivity:

Validation of Health Advisories

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

AIR RESOURCES BOARD
Research Division

Evaluation of Chronic Obstructive Pulmonary Disease Patients for Ozone Sensitivity: Validation of Health Advisories

Final Report

Contract No. A133-123

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California Air Resources Board
Research Division
P.O.Box 2815
Sacramento, California 95812

Prepared by:

Environmental Health Service
Los Amigos Research and Education Institute, Inc.
Downey, California 90242

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Henry Gong, Jr. M.D., Principal Investigator. EHS
Donald F. Tierney, M.D. Principal Investigator, UCLA

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DISCLAIMER

The statements and conclusions in this report are those of the contractor and not necessarily those of the California Air Resources Board. The mention of commercial products, their source or their use in connection with material reported herein is not to be construed as either an actual or an implied endorsement of such products.

NOTE CONCERNING RESEARCH DATA

To allow validation of statistical results presented here and further analysis of experimental data, a computer diskette (IBM PC-compatible) is being submitted with this report. It contains files with all individual physiologic and clinical data discussed in the report, along with necessary documentation of the file structures.

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CONCLUSIONS

We found that small but statistically significant losses in lung function (forced expired volume in one second, FEV_1) resulted from exposure to ozone (O_3) for 4 hr including exercise for 50% of the time at a ventilation rate averaging 20 l/min. These O_3-related losses were not clearly different between volunteers with severe chronic obstructive pulmonary disease (COPD) and otherwise similar healthy older men. However, any measurable loss provokes some concern in patients with severe COPD, because their lung function is greatly diminished by the disease and is impaired additionally by the effects of exercise. Thus the observed lung function losses from O_3 must be considered clinically significant for COPD patients. Prudent medical management would require that they avoid O_3 exposures capable of causing such effects. The experimental O_3 exposures caused no meaningful change in specific airway resistance (SRaw), and small non-statistically-significant increases in reported symptoms. Small equivocally significant decreases in arterial blood oxygen saturation (SaO_2) were more apparent in the middle of O_3 exposure periods than at the end.

Others' previous studies of healthy older adults exposed to O_3 showed responses roughly similar to what we observed (smaller than younger subjects' responses), with roughly similar cumulative O_3 doses delivered by shorter exposures at higher concentrations. The present findings in COPD subjects contrast with previous studies of COPD subjects, which showed no clear lung function changes. The difference is explainable in that previous COPD studies employed shorter exposures with cumulative inhaled doses at least 35-40% lower than ours. Previous studies also may have included predominantly chronic bronchitic subjects, who are suspected to be less responsive than this study's subjects with COPD involving substantial emphysema.

RECOMMENDATIONS

These results reinforce the need for continuing regulatory and advisory efforts to prevent older people (especially those with COPD) from experiencing ambient O_3 exposures as intense as our experimental conditions.

A similar larger-scale study of subjects with COPD might confirm or rule out the "borderline" effects of O_3 on arterial desaturation and symptoms which we observed here. Difficulty in recruiting volunteers with COPD of a predominantly emphysematous type might present a serious obstacle, however. It may be of interest to study people with predominantly bronchitic COPD also. As mentioned in Conclusions, they have been studied previously only at much lower cumulative O_3 dose levels.

INTRODUCTION

Federal and California laws require that the general population and sensitive subgroups must be protected against adverse health effects of air pollution. Implementation involves not only regulating pollution, but also advising the public to take their own protective measures, given that pollution controls so far cannot prevent all known or suspected health effects. This study was commissioned to help validate advisory measures as they relate to one potentially sensitive subgroup - patients with chronic obstructive pulmonary disease (COPD), and one important pollutant - ozone (O_3). Ozone is the pollutant which most often violates health-based air quality standards in California. It is the most potent respiratory toxicant among common pollutants, according to extensive laboratory research on animals and human volunteers [Lippmann, 1992, 1993; McKee, 1994]. After asthma, COPD is the most common chronic disease of the lower respiratory tract, affecting more than 10 million Americans [Redline, 1991]. Usually COPD is associated with a long history of smoking, thus the majority of patients are older men. It is characterized by progressive discomfort and physical disability due to shortness of breath and/or productive cough, and premature death from respiratory insufficiency or associated cardiovascular problems. Patients with COPD are a heterogeneous group including individuals with chronic bronchitis, emphysema, or (most commonly) a mixture of both. Emphysema involves destruction of much of the air-blood interface (the alveoli and associated pulmonary capillaries), limiting the capability to pass oxygen and carbon dioxide across it. This is documentable clinically as a reduction in the diffusing capacity for carbon monoxide (D_LCO) [McLean et al., 1992]. Airways are obstructed due to loss of their normal supporting elastic tissue, which allows them to collapse readily. In chronic bronchitis, airway obstruction relates mostly to excessive mucus secretion. Alveoli and capillaries remain largely intact and D_LCO remains near normal. Emphysemics are likely to be more at risk from O_3 exposure than chronic bronchitics, because emphysemics may be more vulnerable to oxygen desaturation, and more likely to deliver high effective doses of inhaled O_3 to the still-healthy (and therefore overventilated) portions of their lungs. By contrast, bronchitics' excess mucus may consume some O_3 before it has a chance to injure respiratory-tract tissues. Emphysema appears less common than chronic bronchitis, but still affects millions of people in the U.S. [Redline, 1991].

Past controlled exposure studies have shown few or no O_3 effects on lung function or symptoms in volunteers with COPD [Solic et al., 1982; Linn et al., 1982, 1983a; Kehrl et al., 1985]. However, their negative results may have reflected comparatively short exposures and/or a predominance of chronic bronchitic subjects. This study was designed to test more definitively the response of "highest-risk" COPD patients to O_3 exposures representative of the "worst-case" summer pollution episodes in Southern California. Experimental hypotheses were as follows: (1) Men with severe COPD of a predominantly emphysemic type experience respiratory symptomatic and/or physiologic impairment during a "worst-case" O_3 exposure, more so than in a similar exposure to clean air. (2) The aforementioned COPD patients are more responsive to O_3 exposure than healthy men of similar age. To test these hypotheses, subjects were exposed for 4-hr periods to 0.24 ppm O_3 - within the "first-stage-alert" range, less than the maximum hourly average in the most polluted communities but near the maximum for a 4-hr interval. During exposure the subjects were required to exercise 50%

of the time as vigorously as they could over such a prolonged period. Volunteers with COPD were accepted for study only if they had a reduced D_LCO, indicating substantial emphysema.

METHODS

Subject Recruitment and Screening. Male subjects were recruited by referrals from local pulmonologists, by newspaper advertisements, and by invitations to previous research volunteers who met the screening criteria. Ten healthy volunteers and nine with COPD were recruited, passed their screening examinations, and completed both exposures. Table 1 reports their individual screening results including age, size, forced expired volume in one second (FEV_1) and forced vital capacity (FVC) as percentages of age-size-predicted values (%P) [Knudsen et al., 1983], D_LCO %P [Cotes, 1979], and smoking history. All COPD subjects and half the healthy subjects were former smokers (stopped ≥ 2 yr); the remaining healthy subjects had never smoked regularly. All healthy subjects had FVC, FEV_1, and D_LCO well within statistical normal limits for their age and size. All COPD subjects had FEV_1 well below the normal range, and FVC either within the normal range or subnormal but proportionately larger than FEV_1. Originally it was planned to require D_LCO %P < 50 in COPD subjects, but that criterion had to be relaxed because too few volunteers met it. The final COPD subject group averaged 50% of predicted, with a range of 27% to 72%. In addition to the above physiologic measurements, screening included resting and exercise electrocardiograms to rule out clinically significant heart disease and demonstrate exercise tolerance. To rule out asthma, healthy volunteers underwent bronchial reactivity tests with inhalation of increasing doses of methacholine aerosol, employing an abbreviated version of the standard protocol [Chai et al., 1975]. All showed < 20% FEV_1 decrement at the maximum cumulative methacholine dose of ≈200 breath units (a breath unit being one vital capacity breath of aerosol containing one mg/l of methacholine chloride). Bronchial lability tests were not performed on COPD subjects because of safety considerations; and because their low D_LCO, in combination with history and spirometric abnormalities, was considered sufficient to verify their condition.

Exposures. Exposures were performed in the Rancho Los Amigos main environmental chamber [Hackney et al., 1984]. Temperature was set at 24° C and relative humidity at 40% to mimic a mild summer day in a high-O_3 community. (Higher temperatures are more common in ambient O_3 pollution episodes, but a higher experimental temperature would have reduced the subjects' exercise tolerance, causing them to inhale less O_3 and making it more difficult to detect an effect.) Purified air was supplied at a rate of ≈10 changes/hr with no recirculation. For O_3 exposures, the pollutant was generated using a Welsbach industrial type high-voltage-discharge generator fed with chromatographic-grade compressed air. (No appreciable contamination by nitrogen dioxide occurred because the generator was operated near minimum voltage.) Ozone was monitored by a Dasibi ultraviolet photometer with calibration traceable to the South Coast Air Quality Management District's instrument laboratory.

Subjects were exposed two or three at a time, beginning the protocol 15 min apart. Each person performed body plethysmography, spirometry, pulse oximetry, and symptom measurements (see next section), always in clean air, approximately 30 min before exposure began. During exposure he exercised for the first 15 min of each half hour for a total of 4 hr, riding a cycle ergometer during the first, third, etc. periods and walking on a treadmill during the second, fourth, etc. periods. Breathing was unencumbered during all exposures. The target ventilation rate (minute volume) at exercise was the maximum the subject could

comfortably maintain, expected to be about 20-30 l/min. As indicated in Results, typical subjects' actual measured ventilation rates were at the low end of this target range. Each subject's electrocardiogram was monitored continuously throughout exposure via telemetry. Spirometry was performed during the rest period at the end of each hour. Symptoms were recorded at the end of each exercise and rest session. Pulse oximetry and ventilation rate measurements were performed during the latter half of each exercise session. At the end of 4 hr the subject left the chamber. Body plethysmography, pulse oximetry and spirometry measurements were performed as soon as practical thereafter. Spirometry was repeated 30 and 60 min after the subject left the chamber. Just after the 60-min-post spirometric testing, methacholine aerosol challenges were performed on healthy subjects only, using the abbreviated Chai et al. [1975] procedure as in screening examinations. In lieu of methacholine tests, COPD subjects were offered a normal dose of inhaled bronchodilator (albuterol), and those who took the drug had repeat spirometric measurements 15 min later to quantitate their response. Ozone and clean air exposures were performed a minimum of 7 days apart; their order was chosen at random. Neither subjects nor staff members making the health measurements were informed of the exposure order; however, true double-blind conditions were not possible because of the distinctive odor of O_3. COPD subjects who were on respiratory medications were requested to withhold them prior to each exposure for 6 hr to 72 hr, depending on the particular drug's clearance rate, to avoid possible masking of exposure effects. Two subjects were, however, allowed some bronchodilator medication prior to both studies to control their symptoms. Judging from findings in healthy people, this would not likely have blocked their lung function or symptomatic responses to O_3 exposure [Gong et al., 1988].

Due to scheduling constraints, 10 subjects were exposed during a season of high ambient O_3 pollution levels (in August-September 1993). The other 9 were exposed in a lower-pollution season (between November 1993 and early June 1994), as is preferred to minimize intercurrent ambient exposures. Because most subjects lived in the less polluted coastal regions of the Los Angeles Basin, their personal ambient exposures were expected to be low in comparison to experimental O_3 exposures. As indicated in Results, seasonal effects on response to experimental exposures appeared unimportant.

Measures of Response. Spirometric measurements (FVC and FEV_1) were performed using a Stead-Wells low-inertia water-sealed spirometer (W.E. Collins Inc., Braintree, MA) electronically interfaced to a personal computer with data processing software developed in this laboratory [Linn et al., 1990]. This system was checked daily with an electronic flow-volume calibration syringe (Jones Medical Instrument Co., Oak Brook, IL). Specific airway resistance (SRaw) was measured in a pressure-type body plethysmograph. Its volume, flow, and pressure transducers were calibrated daily by applying standard inputs mechanically. Ventilation rates were measured by having subjects breathe through a large-bore one-way valve (Hans-Rudolph, Kansas City, MO) into a Parkinson-Cowan dry gas meter (Carl Poe Co., Houston). Arterial oxygen saturation (SaO_2), i.e. the percentage of hemoglobin in arterial blood which was oxygenated, was measured using a pulse oximeter with finger probe (Nellcor, Inc., Hayward, CA). Symptoms likely to be caused by an inhaled irritant were recorded on a standardized questionnaire and scored according to intensity, as in previous similar exposure studies [Linn et al., 1982, 1983a,b]. Table 2 gives details.

TABLE 1
SUBJECT CHARACTERISTICS

ID	GROUP	AGE	HEIGHT	WEIGHT	FEV1 %P	FVC %P	FEV1/FVC	DLCO %P	PAST SMOKING*
1596	COPD	62	66	179	47	51	73	59	YES
1622	COPD	59	66	154	51	85	44	37	YES
1636	COPD†	68	72.5	178	22	63	27	50	YES
2164	COPD†	64	72	178	14	56	20	27	YES
2168	COPD†	69	70	148	16	42	30	35	YES
2169	COPD†	70	66	149	26	62	33	60	YES
2171	COPD†	71	71	142	17	64	20	56	YES
2190	COPD	65	64	174	49	62	44	68	YES
2196	COPD†	63	70	169	33	83	32	72	YES
COPD MEAN ± S.D.		66 ± 4	69 ± 3	163 ± 15	31 ± 15	63 ± 14	36 ± 15	52 ± 16	[100% X]
2165	HEALTHY	69	70	220	117	100	91	137	NO
2166	HEALTHY	67	69	208	99	94	83	89	NO
2178	HEALTHY	67	72	150.5	107	109	77	137	YES
2179	HEALTHY	64	71	183.5	98	110	69	123	YES
2181	HEALTHY	68	69.5	185	88	86	81	99	NO
2182	HEALTHY	67	67.5	168	104	122	67	128	NO
2183	HEALTHY	63	67	188	85	90	75	100	YES
2184	HEALTHY	60	68.5	196	81	96	67	97	YES
2186	HEALTHY	67	70.5	178	82	77	63	87	YES
2195	HEALTHY	62	66.5	164	103	98	80	147	NO
HLTH. MEAN ± S.D.		65 ± 3	69 ± 2	184 ± 21	97 ± 12	98 ± 13	75 ± 8	114 ± 22	[50% X]

*YES = Ex-smoker, > 1 pack-year lifetime, quit > 2 years ago. NO = never smoked regularly.
†Currently taking respiratory medications -- bronchodilators and/or corticosteroids.

TABLE 2
SYMPTOM SCORING PROCEDURE*

SPECIFIC SYMPTOMS SCORED:

Lower Respiratory Category
- cough
- sputum
- dyspnea
- chest tightness
- wheeze
- substernal irritation

Upper Respiratory Category
- nasal congestion/discharge
- throat irritation

Non-Respiratory Category
- headache
- fatigue
- eye irritation
- miscellaneous (written in by subject)

SCORING FOR EACH SYMPTOM AT EACH TIME OF RECORDING:

0	not present
5	minimal (not noticeable unless asked about)
10	mild (noticeable but not bothersome)
20	moderate
30	severe
40	incapacitating

*At each time of recording, a total symptom score (SS) and subtotals for lower, upper, and non-respiratory categories are determined by summing the scores for specific symptoms.

All the aforementioned data were analyzed using standard commercially available programs (BMDP Statistical Software, Los Angeles) for analysis of variance (anova) and regression. Analytical details are discussed in Results. In most instances an unfavorable effect of O_3 would be manifested as a significant ($P < 0.05$) interaction of the atmosphere and time effects in a repeated-measures anova.

RESULTS

Ventilation Rates. For each subject under each exposure condition, representative exercise ventilation rates were determined as the median of four measurements during four cycle exercise periods, and the median of four measurements during four treadmill walking periods. (Medians were used to avoid the influence of occasional outlying, possibly erroneous, data.) These representative cycling and walking ventilation rates were subjected to anova with repeated measures on subjects, using program BMDP2V [Dixon, 1988]. All subjects' overall mean ventilation rate at exercise was 20 l/min. Means for cycling and walking were 19 and 21 l/min respectively; that difference was statistically significant ($P = 0.01$). Such a difference is expected in subjects who are more accustomed to walking than to cycling - leg muscle fatigue is more of a limiting factor in cycling. Variations in exercise ventilation rate due to clinical status (healthy vs. COPD), atmosphere (O_3 vs. clean air), or interactions among any of the analyzed factors was non-significant ($P > 0.2$). Ventilation rates measured at rest ranged from 7 l/min to more than 15 l/min. The higher values were considered to reflect artifacts of the measuring apparatus, which should be proportionately more important at rest than at exercise. We therefore assumed an overall average resting ventilation rate of 10 l/min. Thus with exercising and resting each for 120 min total, all subjects' estimated average ventilation during an entire exposure was (120 x 10) + (120 x 20) = 3600 l, and their estimated average inhaled dose of O_3 was 3600 x 0.24 = 864 ppm-l (hypothetically, 864 μl of pure O_3 at ambient temperature and pressure).

Spirometry. Figure 1 shows mean FVC and FEV_1 for all 19 subjects as a function of time before and during exposures. Figure 2 shows the mean percentage change in FEV_1 after each hour of exposure, separately for healthy and COPD subjects. Table 3 shows individual FEV_1 measurements before exposure and after 4 hr, and the corresponding percentage changes. The FVC and FEV_1 data were subjected to repeated-measures anova as described above. Absolute changes from preexposure values (ΔFVC, ΔFEV_1) and changes expressed as a percentage of the preexposure value (ΔFVC%, ΔFEV_1%) were analyzed similarly. Huynh-Feldt adjustments were applied when necessary to correct the resulting P values for effects of nonideal data distributions. Table 4 presents statistical results. FEV_1, but not FVC, showed significant unfavorable changes attributable to O_3. Specifically, FEV_1 decreased during O_3 exposure relative to clean air, and this decrement tended to increase with time. As the figures indicate, function tended to improve during the latter part of clean air exposures, partly reversing an initial decline; whereas in O_3, function stayed the same or declined further during the later hours. That pattern was similar for FEV_1 and FVC. The overall FVC difference between clean air and O_3 exposures (the main effect of atmosphere) was statistically significant ($P < 0.0005$). However, that result is not clear evidence of an O_3 effect, because the function decrement was present before exposure as well as during exposure. The key statistical result, the atmosphere-time interaction, was not significant for FVC ($P = 0.40$). For FEV_1, the atmosphere-time interaction was significant whether the analysis dealt with absolute data ($P = 0.008$) or with percentage changes from the preexposure baseline ($P = 0.036$). Interestingly, the O_3 effect was not significantly different in healthy and COPD subjects - the interaction of atmosphere, time, and clinical status was always non-significant. However, the mean percentage loss in FEV_1 attributable to O_3 was more than twice as large in COPD subjects as in

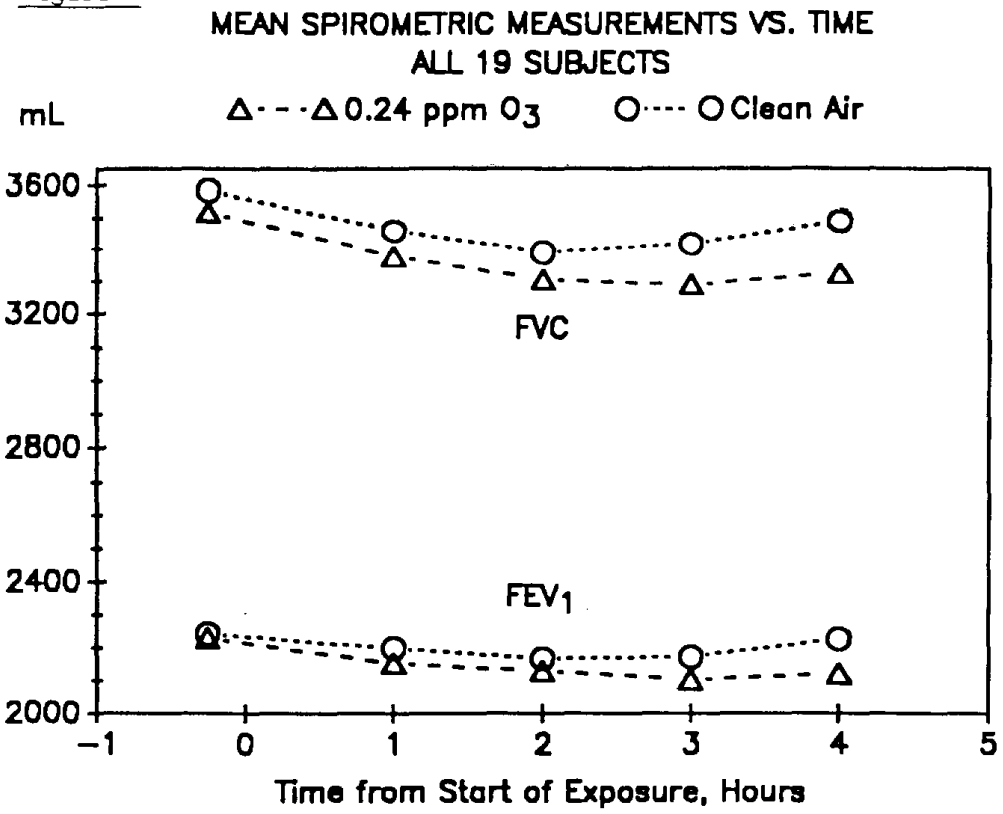
healthy subjects (Table 3), and might have achieved statistical significance had the COPD group been larger (see below).

The absolute FEV_1 loss attributable to O_3 averaged larger in healthy subjects (112 ml) than in COPD subjects (72 ml), even though it represented a much smaller percentage of the healthy subjects' much larger baseline FEV_1. As expected, the main effect of clinical status (the overall difference in function between healthy and COPD subjects) was highly significant for both FVC and FEV_1 ($P < 0.0001$). The interaction of clinical status and time was significant for both function measures ($P < 0.05$), showing that the exposure/exercise protocol induced more function loss in COPD subjects than in healthy subjects, whether or not O_3 was present. As Table 3 indicates, the percentage FEV_1 loss (mean ± s.d.) after the full 4 hours' O_3 exposure - including the exercise effect as well as the O_3 effect - was about 19% ± 11% in COPD subjects, versus 2% ± 4% in healthy subjects - a highly significant difference ($P = 0.0002$). That response was reanalyzed to assess differences between subjects exposed in high-pollution and lower-pollution seasons, along with differences between COPD and healthy subjects. The COPD effect remained highly significant. The season effect was non-significant ($P > 0.2$), and mean FEV_1 losses were larger in the high-O_3 season than the low-O_3 season (20% vs. 16% for COPD, 4% vs. 0% for healthy subjects). Thus it seems very unlikely that intercurrent ambient O_3 exposures attenuated the response of subjects whose laboratory exposures took place during the high-pollution season.

Figure 3 presents data from Table 3 graphically, showing individuals' percentage changes of FEV_1 during both exposure studies. Subjects with a more negative change in O_3 are considered "responders"; their individual graphs are labeled on the right. Subjects with a more negative change in clean air are considered "nonresponders"; their individual graphs are labeled on the left. Six of 9 COPD subjects and 8 of 10 healthy subjects were responders. When either clinical subgroup was analyzed by itself, the mean excess FEV_1 loss in O_3 relative to clean air (about 8% in COPD, 3% in healthy subjects) was not statistically significant. That is not surprising since statistical power is markedly lower with separate samples of 9 or 10 than with a combined sample of 19 subjects. As discussed later, attempts to correlate individuals' FEV_1 responses to O_3 with their responses measured in other ways, or with their symptom status prior to exposure, were not successful.

The overall impression from the spirometry data is that both healthy and COPD subjects experienced lung dysfunction after 4 hr exposure to O_3 with intermittent exercise. In the healthy older subjects these O_3-associated losses represented a small proportion of their functional reserves and may not have been clinically important. In the COPD subjects, however, even small O_3-associated losses are of more concern. Their functional reserves are small at best, due to their underlying disease. Furthermore, for most of them the effect of O_3 exacerbated an acute function loss due to exercise.

Figure 1


MEAN SPIROMETRIC MEASUREMENTS VS. TIME
ALL 19 SUBJECTS
mL
Δ- - -Δ 0.24 ppm O3
O···· O Clean Air
3600
3200
2800
2400
2000
FVC
FEV1
−1
0
1
2
3
4
5
Time from Start of Exposure, Hours

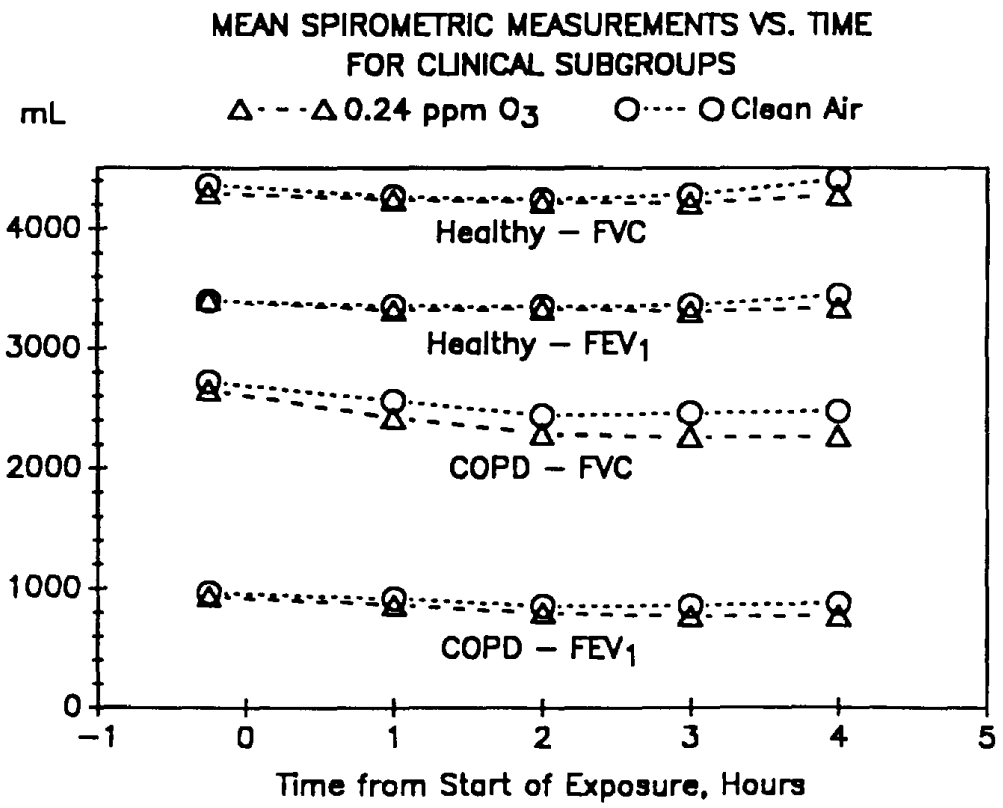
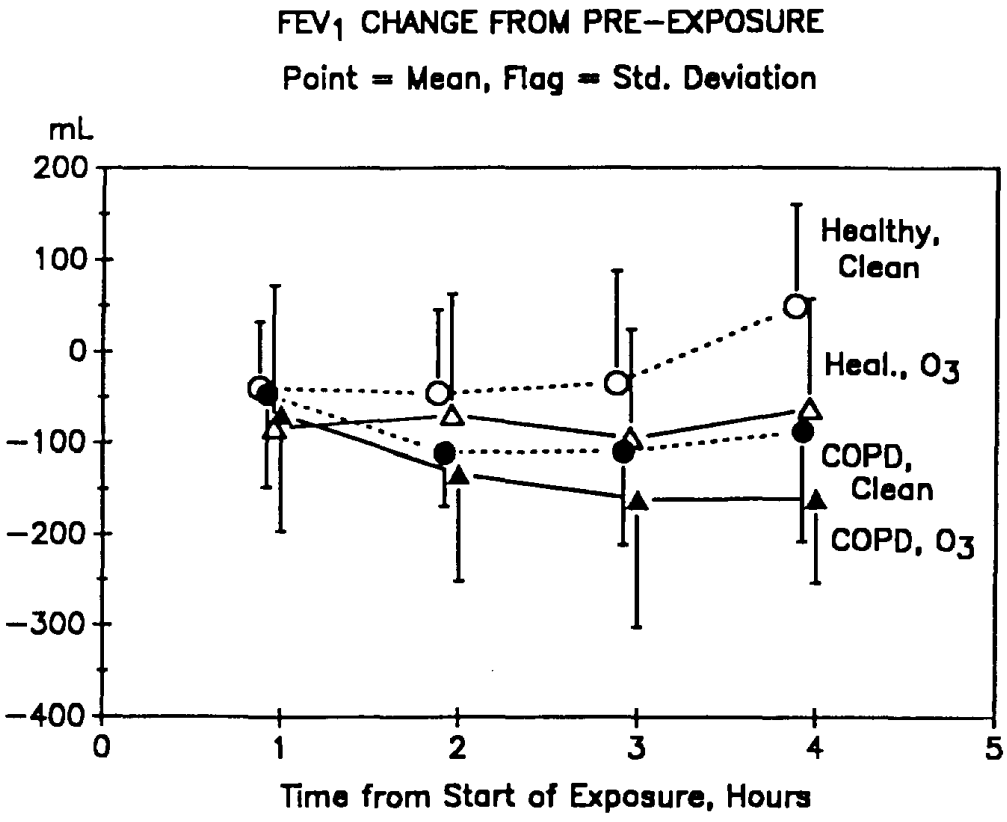

MEAN SPIROMETRIC MEASUREMENTS VS. TIME
FOR CLINICAL SUBGROUPS
mL
Δ- - -Δ 0.24 ppm O3
O···· O Clean Air
4000
3000
2000
1000
0
Healthy — FVC
Healthy — FEV1
COPD — FVC
COPD — FEV1
−1
0
1
2
3
4
5
Time from Start of Exposure, Hours

Figure 2


FEV1 CHANGE FROM PRE-EXPOSURE
Point = Mean, Flag = Std. Deviation
mL
200
100
0
−100
−200
−300
−400
0
1
2
3
4
5
Healthy, Clean
Heal., O3
COPD, Clean
COPD, O3
Time from Start of Exposure, Hours

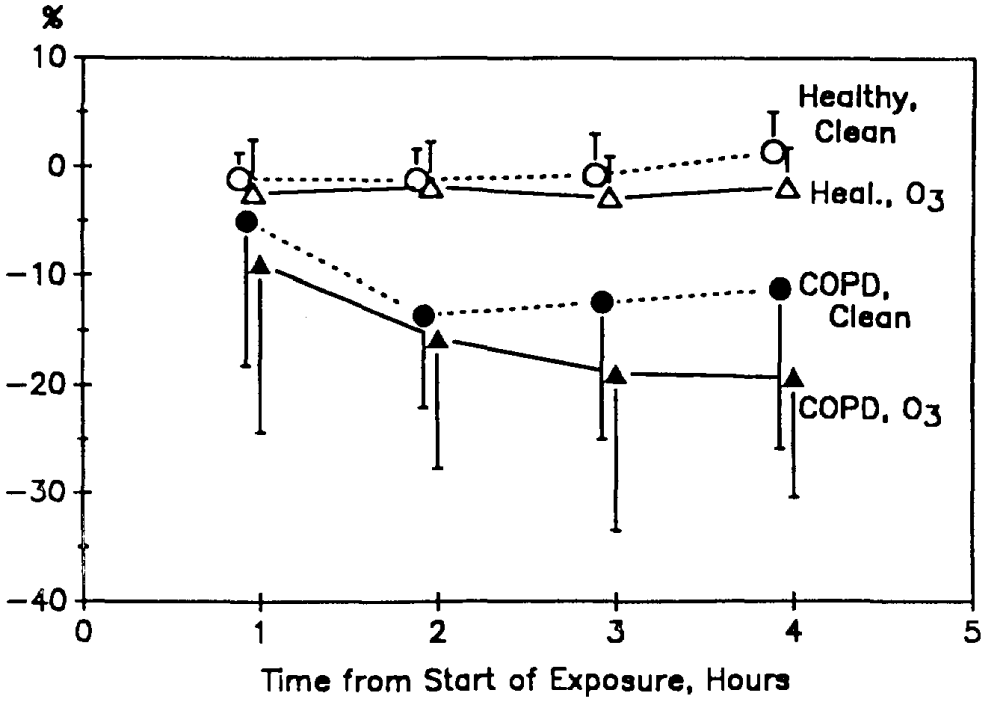

FEV1, % CHANGE FROM PRE-EXPOSURE
Point = Mean, Flag = Std. Deviation
%
10
0
−10
−20
−30
−40
0
1
2
3
4
5
Healthy, Clean
Heal., O3
COPD, Clean
COPD, O3
Time from Start of Exposure, Hours

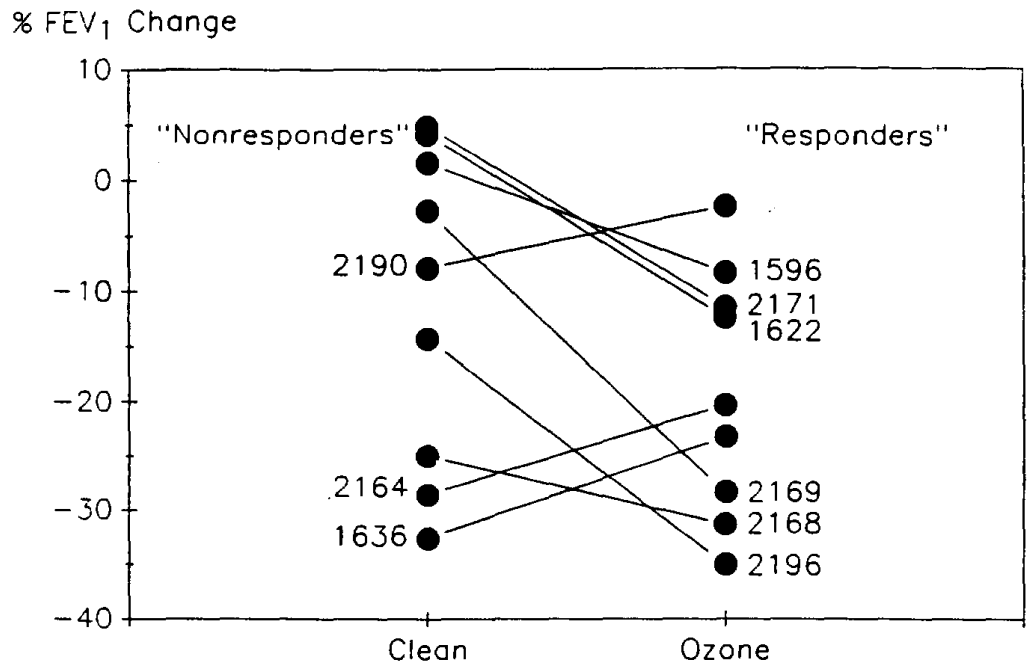
TABLE 3
INDIVIDUAL SUBJECTS' FEV_1 BEFORE AND AFTER 4 HR EXPOSURE
TO OZONE AND CLEAN AIR, AND PERCENTAGE CHANGES

		0.24 PPM OZONE			CLEAN AIR		
ID	GROUP	PRE-EXP.	END-EXP.	% CHANGE	PRE-EXP.	END-EXP.	% CHANGE
1596	COPD	1.31	1.20	-8.40	1.31	1.33	1.53
1622	COPD	1.37	1.20	-12.41	1.47	1.53	4.08
1636	COPD	1.03	0.79	-23.30	0.92	0.62	-32.61
2164	COPD	0.59	0.47	-20.34	0.70	0.50	-28.57
2168	COPD	0.51	0.35	-31.37	0.60	0.45	-25.00
2169	COPD	0.74	0.53	-28.38	0.72	0.70	-2.78
2171	COPD	0.61	0.54	-11.48	0.62	0.65	4.84
2190	COPD	1.25	1.22	-2.40	1.51	1.39	-7.95
2196	COPD	0.97	0.63	-35.05	0.84	0.72	-14.29
COPD MEAN		0.93	0.77	-19.24	0.97	0.88	-11.19
COPD S.D.		0.33	0.35	11.22	0.37	0.42	14.55
2165	HEALTHY	3.98	4.05	1.76	4.11	4.12	0.24
2166	HEALTHY	3.45	3.36	-2.61	3.49	3.45	-1.15
2178	HEALTHY	4.07	4.00	-1.72	3.97	4.13	4.03
2179	HEALTHY	4.00	3.80	-5.00	3.87	4.01	3.62
2181	HEALTHY	3.06	2.83	-7.52	3.03	2.87	-5.28
2182	HEALTHY	3.25	3.27	0.62	3.32	3.39	2.11
2183	HEALTHY	3.05	2.93	-3.93	2.95	2.89	-2.03
2184	HEALTHY	3.17	3.04	-4.10	3.14	3.24	3.18
2186	HEALTHY	2.70	2.66	-1.48	2.73	2.93	7.33
2195	HEALTHY	3.25	3.11	-4.31	3.32	3.39	2.11
HEALTHY MEAN		3.40	3.34	-1.91	3.39	3.44	1.42
HEALTHY S.D.		0.47	0.49	3.61	0.46	0.49	3.58

TABLE 4
RESULTS OF ANALYSES OF VARIANCE ON SPIROMETRIC DATA (P VALUES)

SOURCE OF VARIATION	VARIABLE AND TIMES OF MEASUREMENT USED IN ANOVA										
	FVC 0, 1,2,3,4 hours	FEV_1 0, 1,2,3,4 hours	ΔFVC 1,2,3,4 hours	ΔFEV_1 1,2,3,4 hours	ΔFVC % 1,2,3,4 hours	ΔFEV_1 % 1,2,3,4 hours	ΔFVC 4 hours	ΔFEV_1 4 hours		ΔFEV_1 % 1,2,3,4 hours COPD	ΔFEV_1 % 1,2,3,4 hours Healthy
Clinical Status (C)	.000	.000	.033	.148	.021	.003	.015	.010			
Atmosphere (A)	.000	.002	.397	.049	.189	.060	.206	.009 *		.180	.093
C x A Interaction	.082	.417	.350	.714	.175	.353	.637	.533			
Time (T)	.002	.000	.242	.012	.220	.003				.006	.024
C x T Interaction	.032	.008	.166	.001	.139	.001					
A x T Interaction	.404	.008 *	.332	.027 *	.273	.036 *				.217	.124
C x A x T Interact.	.736	.928	.905	.854	.906	.671					

*Indicates statistically significant unfavorable effect of O_3 exposure, relative to clean air control.



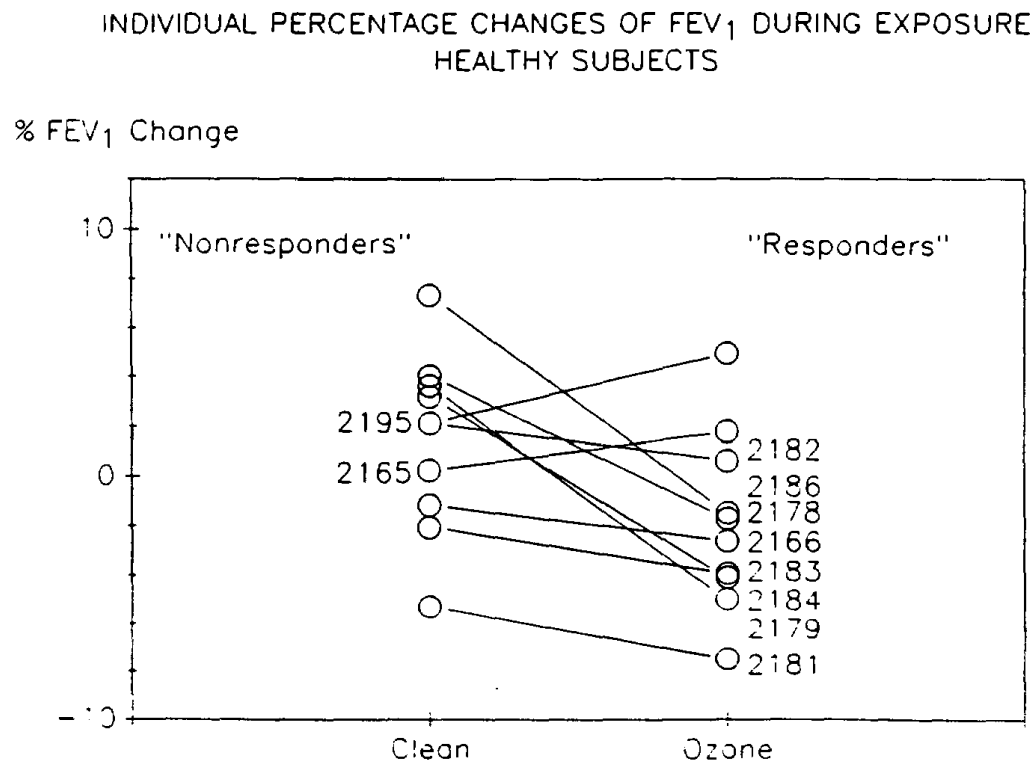


Figure 3. Individual FEV_1 responses. "Nonresponders" (individual graphs labeled on left) had more negative change in clean air. "Responders" (labeled on right) had more negative change in ozone. See Table 3 also.

Airway Resistance. Figure 4 shows mean SRaw at preexposure (baseline) measurements and the mean and standard deviation of change pre- to postexposure, separately for healthy and COPD subjects. Table 5 gives results of repeated-measures anova on SRaw data. As expected, SRaw was significantly higher overall in COPD subjects ($P < 0.0001$ for main effect of clinical status). The overall effect of time, and the interaction of time with clinical status, were also significant ($P < 0.01$). The healthy subjects showed little change in SRaw pre- to postexposure; thus the significance of main effect as well as the interaction depended mostly on the COPD subjects' increase in SRaw during exposure. That response is wholly attributable to exercise or other stresses inherent in the experimental protocol, since it was not significantly different in clean air and O_3 ($P \geq 0.40$ for interactions of atmosphere with other effects).

Blood Oxygenation. Some measurements of SaO_2 were missing or unsatisfactory because of difficulty in getting a stable reading from the pulse oximeter's fingertip probe during exercise. Because of that problem, two different statistical methods were applied to maximize the chance of detecting an O_3 effect. First, trends of SaO_2 during exposure were determined for each individual. Trends were calculated as slopes from linear regressions of SaO_2 vs. time, separately for cycling and walking exercise. A repeated-measured anova was performed to test whether the mean slope differed significantly between clean air and O_3 exposures, between walking and cycling, or between healthy and COPD subjects. Second, an unbalanced repeated-measures anova (program BMDP5V) was performed on SaO_2 data using a maximum-likelihood-estimation procedure and assuming a general autoregressive structure, i.e. assuming in general that measurements are more alike if they are closer together in time [Dixon, 1988]. Both analyses implicitly assumed that the pattern of missing SaO_2 data was unrelated to the pattern of O_3 effects (if any). That assumption is supported by the observation that the incidence of missing data during exercise was 3% to 4% both in clean air and in O_3 studies.

The first analysis showed no significant variation in the time trend (slope) of SaO_2 due to atmosphere, mode of exercise, or clinical status. The overall mean slope was not significantly different from zero; i.e., SaO_2 did not change meaningfully with increasing duration of exposure according to this analysis. The atmosphere effect approached significance ($P = 0.053$) in the "wrong" direction - the change of SaO_2 with time was more negative in clean air than in O_3. Figure 5 and Table 6 show results of the second analysis, estimating effects of clinical status, time, atmosphere, and interactions among those factors, and summing those estimates to predict mean SaO_2 at each time point for each subgroup. Overall, the COPD subjects showed a 3.0% lower estimated mean SaO_2 than healthy subjects ($P < 0.0005$). All subjects' mean SaO_2 was estimated as 0.24% lower during O_3 exposures than during clean air exposures (not significant, $P = 0.21$). The variation over time and the interaction of time with clinical status were highly significant ($P < 0.0005$), with a complex pattern. Exercise appeared to increase healthy subjects' SaO_2, but to decrease COPD subjects' SaO_2. Such exercise-related decreases are common, though not universal, in people with COPD [Wagner, 1991]. The significant atmosphere-time interaction ($P = 0.014$) suggested a transient O_3-related decrement in the intermediate hours of exposure. A transient change would not give rise to negative slopes in the first analysis; thus a non-significant O_3 effect in the first analysis and a significant O_3 effect in the second analysis are not

inconsistent. In any event, changes in SaO_2 during exposure studies, whether or not attributable to O_3, were small and not necessarily of medical significance.

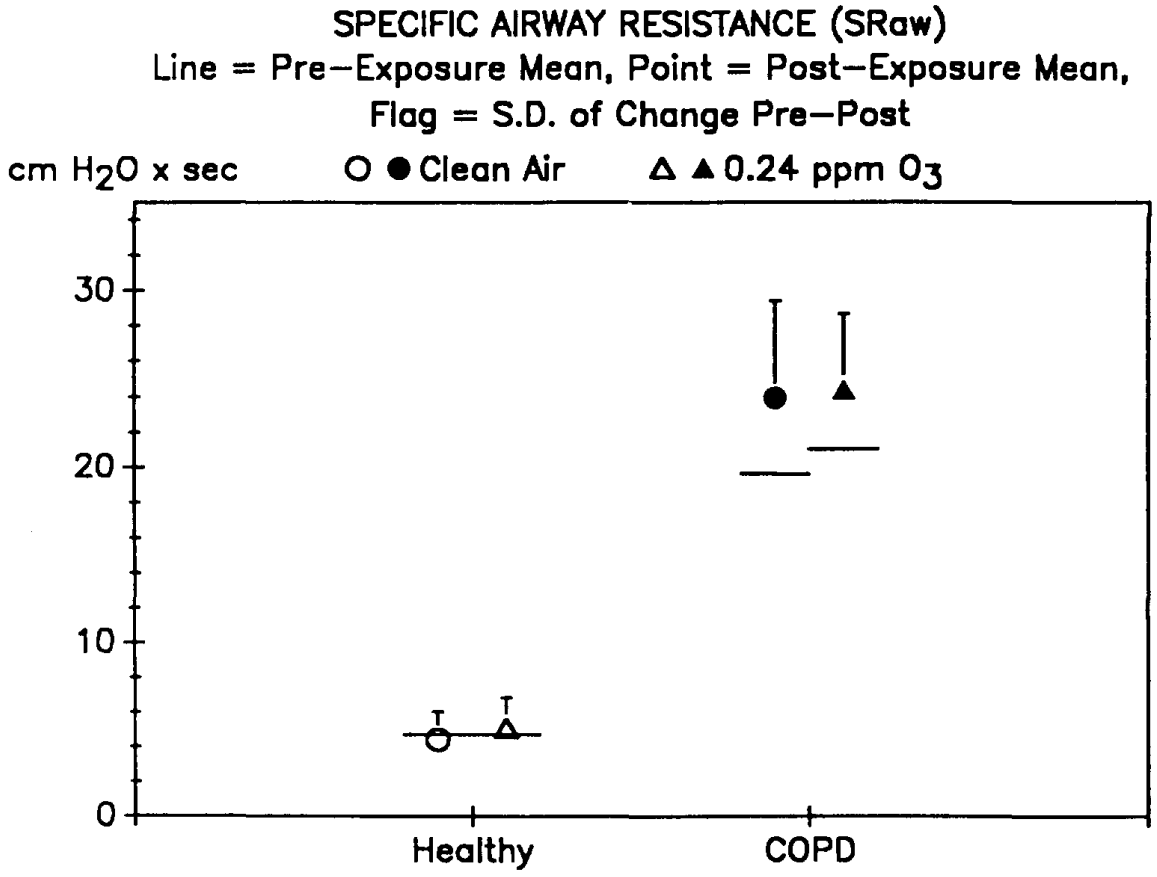


Figure 4. Specific airway resistance. Units of measurement are pressure per unit flow, times lung volume at which measurement is made, i.e. centimeters of water (pressure) per liter per second (flow) times liters (lung volume),or cm H_2O x sec. Statistical limits of normal are approximately 2 to 8 cm H_2O x sec [Cotes, 1979].

TABLE 5
ANALYSES OF VARIANCE ON SPECIFIC AIRWAY RESISTANCE AND SYMPTOM SCORE (P VALUES)

SOURCE OF VARIATION	VARIABLE AND TIMES OF MEASUREMENT USED IN ANOVA									
	SRaw 0,4 hours	ΔSRaw % 4 hours	Lower Resp. SS 0, 1,2,3,4 hours	Upper Resp. SS 0, 1,2,3,4 hours	Non-Resp. SS 0, 1,2,3,4 hours	Total SS 0, 1,2,3,4 hours	Lower Resp. ΔSS 4 hours	Upper Resp. ΔSS 4 hours	Non Resp. ΔSS 4 hours	Total ΔSS 4 hours
Clinical Status (C)	.000	.253	.000	.003	.005	.000	.099	.190	.185	.093
Atmosphere (A)	.420	.374	.519	.660	.972	.300	.161	.450	.567	.226
C x A Interaction	.698	.666	.329	.780	.972	.340	.763	.945	.413	.697
Time (T)	.007		.007	.319	.002	.002				
C x T Interaction	.007		.026	.011	.013	.003				
A x T Interaction	.907		.208	.352	.659	.216				
C x A x T Interact.	.465		.629	.605	.276	.507				

TABLE 6
RESULTS OF UNBALANCED REPEATED-MEASURES ANALYSIS OF VARIANCE ON SaO_2 DATA (P VALUES FROM WALD CHI-SQUARE SIGNIFICANCE TESTS)

FACTOR	P
Clinical status (C)	.000
Atmosphere (A)	.210
Time (T)*	.000
C x E interaction	.728
C x T interaction	.000
E x T interaction	.014†
C x E x T interaction	.311

*SaO_2 measurements at 10 different times were included in anova: time 1 - preexposure at rest; times 2, 4, 6, 8 - bicycle exercise periods; times 3, 5, 7, 9 - treadmill exercise periods; time 10 - postexposure at rest. Difference between exercise and rest was not tested specifically here, but was found significant in separate analyses ($P < 0.01$).

†Indicates significant unfavorable effect of O_3 relative to clean air.

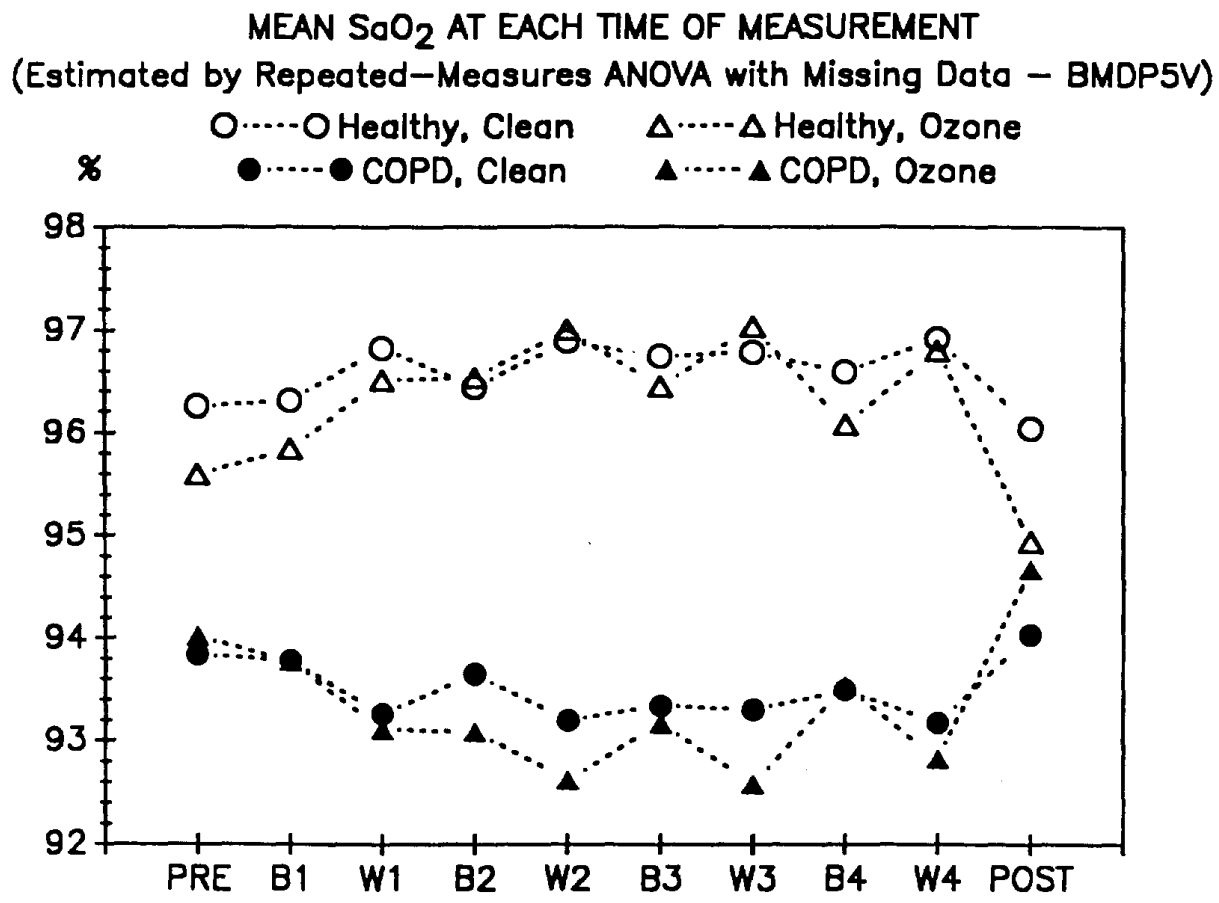


Figure 5. Mean arterial oxygen saturation as a function of time, for each clinical subgroup. Pre and post measurements at rest, others at exercise. B1 = bicycle exercise in 1st hour of exposure, W1 = treadmill walking exercise in 1st hour, B2 = bicycle exercise in 2nd hour, etc. Bicycle exercise always preceded walking exercise in a given hour.

Symptom Reports. Figure 6 shows mean total symptom scores (SS) preexposure, in the last exercise period of each hour during exposure, and immediately postexposure, for each clinical subgroup. Those data were subjected to repeated-measures anova. Table 5 summarizes anova results for total symptom scores and for lower-, upper-, and non-respiratory subtotals. As expected, COPD subjects were significantly more symptomatic than healthy subjects ($P < 0.0005$ for main effect of clinical status on total score). Symptoms increased during exposure ($P < 0.0005$ for main effect of time), more so in COPD subjects than in healthy subjects ($P < 0.0005$ for clinical status-time interaction). Ozone had no significant effect on total symptom scores ($P > 0.2$ for main effect of atmosphere and related interactions). Conclusions were much the same if only the preexposure and postexposure total scores were analyzed. Although the difference was not significant, total score increases pre- to postexposure were larger in O_3 than in clean air, by an average of 10.5 points in COPD subjects and 5.5 points in healthy subjects. As Table 2 indicates, an increase of 5 points is "minimal" or barely perceptible. An increase of 10 points may represent the development of one extra "mild" symptom (noticeable but not bothersome), or an exacerbation of a preexisting symptom from "mild" to "moderate" or from "moderate" to "severe".

Of the average 23-point increase in total symptom score during O_3 exposures of COPD subjects, lower respiratory symptoms accounted for 14, non-respiratory symptoms for 7, and upper-respiratory symptoms for 2. Healthy subjects' average increase of 6 points during O_3 exposures reflected an increase of 5 for lower-respiratory symptoms, an increase of 2 for non-respiratory symptoms, and a decrease of 1 for upper-respiratory symptoms.

Separate analyses were performed for particular symptom scores thought to be highly specific to O_3 effects or to COPD, namely cough, substernal irritation, dyspnea, wheeze, and fatigue. None of those symptoms showed a significant difference between O_3 and clean air, in terms of its score change during 4 hours of exposure. Dyspnea and fatigue showed significantly ($P < 0.05$) larger increases during exposure in COPD subjects than in healthy subjects, regardless of atmosphere. Cough, substernal irritation, and wheeze showed no significant overall increases during exposures and no significant differences between healthy and COPD subjects. Fatigue showed the closest approach to a significant O_3 effect: COPD subjects' fatigue score increased an average of 8 points during O_3 exposures, compared to 3 points during clean air studies.

Bronchial Reactivity in Healthy Subjects. Percentage loss in FEV_1 during methacholine aerosol challenges performed one hour after exposures ceased (mean ± standard deviation) was 10.2 ± 6.8 following O_3, and 8.9 ± 8.6 following clean air. Subjects' maximum doses of methacholine after clean air and O_3 were similar but not always the same. Accordingly, significance of the difference was tested by repeated-measures analysis of covariance (program BMDP2V) with cumulative dose of methacholine as the covariate. The increase in response to methacholine following O_3 exposure proved to be non-significant ($P = 0.18$). A different test for an O_3 effect was performed by calculating dose-response slopes (DRS) according to the method of O'Connor et al. [1987], modified in that the initial FEV_1 measurement in the challenge procedure was used as the baseline, rather than the measurement following saline aerosol inhalation. The DRS (in % FEV_1 loss per

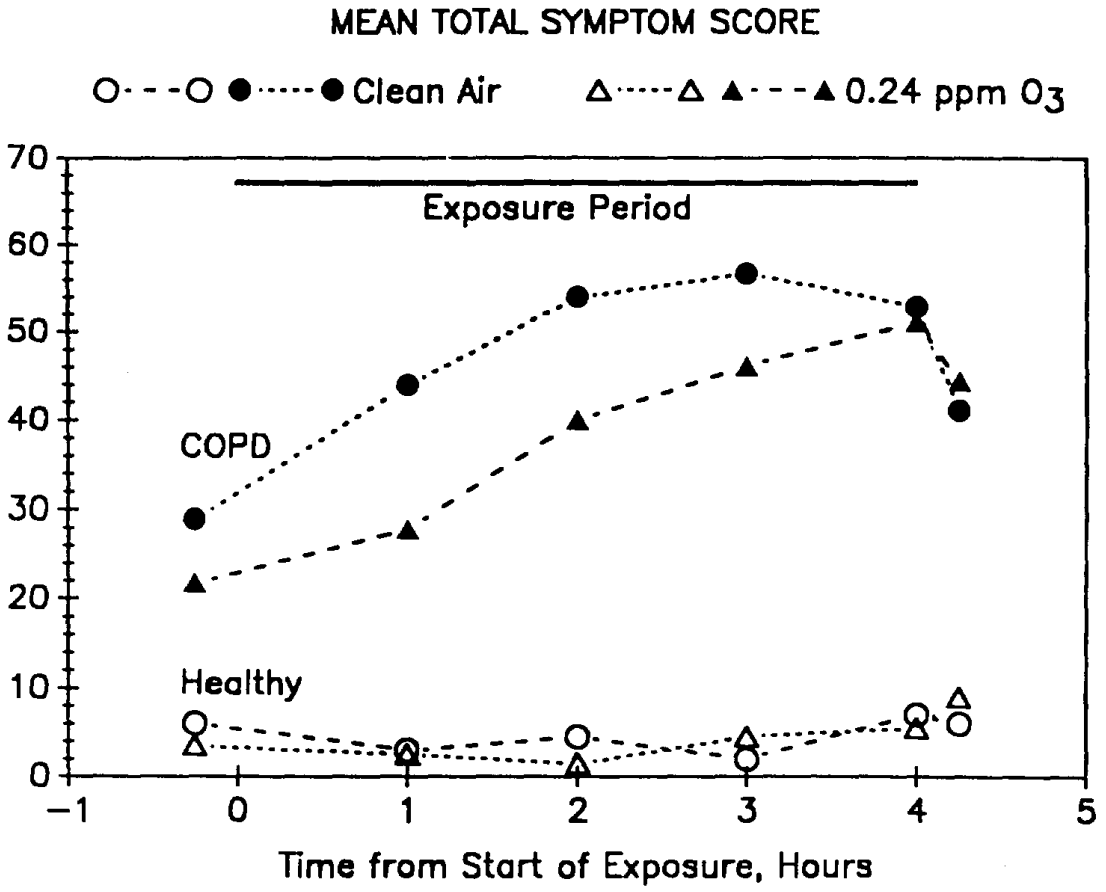


Figure 6. Mean total symptom score as a function of time, for each clinical subgroup. See text for details of symptom responses. Initial and final scores taken at rest, others taken at exercise.

breath unit of methacholine, mean ± s.d) was 0.054 ± 0.035 following O_3 and 0.064 ± 0.087 following clean air. That difference was not significant by repeated-measures anova (P = 0.67). Thus both statistical tests agreed that O_3 exposures did not meaningfully increase healthy subjects' nonspecific bronchial reactivity, in comparison with clean air exposures.

Bronchodilator Response in COPD Subjects. Seven COPD subjects (excluding 2171 and 2190) elected to take a normal dose of bronchodilator (albuterol aerosol) at the conclusions of both their regular exposure protocols. Their forced expiratory function was remeasured 15 minutes after these drug administrations. Mean FEV_1 increased from 0.74 l post-exposure to 0.91 l post-bronchodilator on O_3 days, and from 0.84 l post-exposure to 0.98 l post-bronchodilator on clean air days. The difference between atmospheres was not significant ($P > 0.10$ for atmosphere effect and atmosphere-time interaction, by anova). The time effect, i.e. the increase in FEV_1 after bronchodilator use regardless of atmosphere, was significant (P = 0.02 by anova). Part of that increase may reflect spontaneous reversal of the effects of exercise and/or O_3, rather than a response to the bronchodilator drug.

Interrelationship of Different Response Measures. Relationships among FEV_1, SRaw, and total symptom score changes in response to exposure were explored by determining pairwise correlation coefficients (Pearson's r), and also by analyses of covariance with FEV_1 change as the dependent variable and another variable as a covariate. These analyses employed programs BMDP1R and BMDP2V. Their results showed that different types of response were uncorrelated or weakly correlated. That is, one measure of response to O_3 did not help to predict others; individuals with large FEV_1 losses did not necessarily show large symptom increases or SRaw increases. A similar analysis was performed to test the relationship between the severity of symptoms prior to exposure and the response to O_3 (measured as FEV_1 change). Unusually intense symptoms before exposure might indicate a state of high susceptibility to O_3-induced airway dysfunction. On the other hand, more symptomatic individuals might be more inclined to limit their exercise or alter their breathing patterns to reduce their effective doses of O_3 and thus limit their lung-function responses. Neither possibility was supported by the analysis results: pre-exposure total symptom score was not significantly correlated with FEV_1 loss during exposure. Because of the small subject population, all the analyses mentioned in this paragraph had low statistical power. Their negative results do not necessarily rule out meaningful relationships among the different measures of O_3 response.

DISCUSSION

The subjects of this study, considered as a group, showed unequivocal temporary lung dysfunction (FEV_1 losses) resulting from experimental O_3 exposures. Ozone exposure did not significantly affect subjects' airway resistance or symptomatology, and affected oxygenation of their blood slightly and transiently if at all. Because of the small number of subjects and the small magnitude of response, statistical comparisons between the healthy and COPD subgroups have low power to detect meaningful differences. It appears, however, that the two subgroups were roughly equally susceptible to O_3-induced lung dysfunction in terms of their average FEV_1 losses measured in milliliters. If losses were expressed as a percentage of preexposure FEV_1, the two groups still showed no statistically significant difference but the loss appeared clinically more significant in subjects with COPD, as discussed further below.

The findings of FEV_1 loss support and extend others' previous findings in O_3 exposures of subjects with COPD [Solic et al., 1982; Linn et al., 1982, 1983a; Kehrl et al., 1985] and healthy older adults [Drechsler-Parks et al., 1987; Bedi et al., 1989]. Healthy older adults have shown generally less lung-function response than younger healthy subjects. The Drechsler-Parks and Bedi studies showed FEV_1 decrements roughly similar to those of our healthy subjects, at roughly similar cumulative inhaled doses. Compared to our subjects, Drechsler-Parks' and Bedi's subjects breathed higher O_3 concentrations for shorter times. The previous studies of COPD subjects showed no clear or meaningful lung function changes. That is not surprising, given that we found rather small decrements in spirometric performance attributable to O_3, and previous studies employed shorter exposures with cumulative inhaled doses at least 35-40% lower than ours. Previous studies also may have included predominantly chronic bronchitic subjects, who are likely to be less responsive than our emphysemic subjects (see Introduction). Thus it appears that O_3 can impair lung function under the "worst-case" ambient exposure conditions combining prolonged high ambient concentrations, highly susceptible subjects, and frequent exercise. Earlier studies of people with COPD have not clearly shown this impairment because their exposure conditions were less than "worst case".

In terms of public health, these findings are partly reassuring in that we found no marked symptoms or impairment of blood oxygenation attributable to O_3 exposures, either in the healthy subjects or in those with COPD. Nevertheless, the finding of significant lung function loss due to O_3 raises the possibility that other harmful effects may occur at "worst-case" exposure levels, even if they are not clinically apparent in older adults with or without COPD. Potentially damaging lung inflammation may occur at the same exposure concentrations that induce lung function decrements, although individual inflammatory responses apparently do not correlate with individual physiologic responses [Scannell et al., 1994; Weinmann et al., 1994]. In COPD subjects especially, any measurable loss related to O_3 must provoke concern, given that their functional reserve is very limited because of the underlying disease, and more function is temporarily lost because of exercise. Thus, the spirometric decrements due to O_3 should be considered clinically significant. Most physicians would advise their COPD patients to avoid any pollution exposures (as well as other stresses) which might provoke such responses.

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SYMBOLS AND ABBREVIATIONS

D_LCO	diffusing capacity of the lungs for carbon monoxide
DRS	dose-response slope (a measure of bronchial reactivity to a challenge with inhaled methacholine aerosol)
FEV_1	forced expired volume in 1 second (first second of a maximal forced expiration)
ΔFEV_1	change in FEV_1 relative to pre-exposure measurement
FVC	forced vital capacity (volume of a maximum breath forced out)
ΔFVC	change in FVC relative to pre-exposure measurement
O_3	ozone
P	probability of non-chance difference in statistical comparison
%P	percentage of published predicted value for physiologic test
SaO_2	arterial blood oxygen saturation (percentage of oxyhemoglobin)
SRaw	specific airway resistance (product of airway resistance and the lung volume at which it is measured)
SS	symptom score
ΔSS	change in symptom score relative to pre-exposure measurement