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Respiratory Responses to Exposures With Fine Particulates and Nitrogen Dioxide in the Elderly With and Without COPD

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Elderly people, with and without chronic obstructive pulmonary disease (COPD), may be susceptible to particulate matter (PM) air pollution. However, the respiratory impacts of inhaled PM combined with copollutant(s) in controlled exposure studies are unclear and warrant investigation since exposures to PM–gas mixtures constitute realistic scenarios. Thus, we exposed 6 healthy subjects and 18 volunteers with COPD (mean age 71 yr) on separate days to (a) filtered air (FA); (b) 0.4 ppm NO_2; (c) concentrated ambient particles (CAP), predominantly in the fine ($PM_{2.5}$) size range, at concentrations near 200 $\mu g/m^3$; and (d) CAP and NO_2 together. Each 2-h exposure included exercise for 15 min every half hour. Most respiratory responses, including symptoms, spirometry, and total and differential counts of induced sputum cells, showed no statistically significant responses attributable to separate or combined effects of CAP and NO_2. However, maximal mid-expiratory flow and arterial O_2 saturation (measured by pulse oximetry) showed small but statistically significant decrements associated with CAP, greater in healthy than COPD subjects. CAP exposure was also associated with decreased percentages of columnar epithelial cells in sputum. The results suggest that the respiratory effect of the PM–NO_2 mixture may be primarily PM driven since coexposure to NO_2 did not significantly enhance the responses. In conclusion, older adults exposed to urban fine particles may experience acute small-airways dysfunction with impaired gas exchange. Healthy subjects appear more susceptible, suggesting that the respiratory effect may be related to efficient penetration and deposition of inhaled toxic particles in distal small airways. More clinical investigation of the elderly population is warranted.

Combustion-related primary air pollutants, which include fine particulate matter (PM) and oxides of nitrogen (NOx), are consistently associated with epidemiologic evidence of harm to health, in that cardiorespiratory morbidity and mortality rise with pollutant concentrations in a variety of urban areas (U.S. Environmental Protection Agency, 1996; American Thoracic Society, 1996; Vedal, 1997; Pope, 2000; Dockery, 2001). Particulate matter has been the primary target of recent air quality

regulatory efforts to improve public health. However, the effects of PM exposure are controversial (Vedal, 1997; Green et al., 2002; Green & Armstrong, 2003) because of the limitations of epidemiological and toxicological evidence and the difficulty in identifying a specific pollutant or interaction between coexisting pollutants as a cause of health effects.

Human exposure studies using ambient particle concentrators have the potential to resolve some of these issues (Sioutas et al., 1997; Ghio et al., 2000; Gong et al., 2003). They allow reasonably well-controlled exposures of volunteers to high concentrations of actual ambient pollution particles, to which controlled concentrations of pollutant gases can be added to produce realistic mixtures. The experiments can be conducted in laboratory settings where detailed measurements of exposures and cardiorespiratory responses are possible. Initial investigations have provided suggestive, if not definitive, evidence of pathobiological responses to concentrated ambient particles (CAP) consistent with epidemiological findings (Ghio et al., 2000; Petrovic et al., 2000; Gong et al., 2003; Devlin et al., 2003). We recently reported that CAP exposures of elderly volunteers under conditions similar to "worst-case" ambient exposures ($\sim 200 \ \mu g/m^3$ for 2 h with intermittent exercise) elicited small effects on arterial oxygenation, hematology, heart-rate variability, and ectopic heartbeats. Surprisingly, effects were more noticeable in healthy individuals than in those with COPD (Gong et al., 2004). In the one study of particle-gas interactions published to date, endothelial dysfunction in healthy humans was measured after exposures to combined CAP and ozone at realistic concentrations (Brook et al., 2002). Data from comparable separate tests of CAP or ozone alone exposures are not yet available, so it is unclear whether the circulatory finding represents a true interactive effect or is due to potent effects of a single pollutant per se.

Nitrogen dioxide (NO_2) appears less toxic than ozone in most animal and human inhalation studies (American Thoracic Society, 1996). Most human exposure studies have shown little effect of NO_2 per se, although one study of elderly volunteers reported decrements in lung function among a subgroup with relatively mild chronic obstructive pulmonary disease (COPD) during 4-h exposures to 0.3 ppm (Morrow et al., 1992). Airway inflammation has been found in healthy volunteers exposed to occupational concentrations (within an order of magnitude of ambient levels) of NO_2 alone (Blomberg et al., 1999) and to diesel exhaust containing NO_2 and related combustion particles (Salvi et al., 1999). Epidemiological evidence also frequently points to NO_2 as a risk factor for cardiorespiratory morbidity. In Los Angeles, NO_2 covaries closely with PM pollution and both pollutants are more consistently associated with daily hospital admissions than is ozone (Linn et al., 2000). In Toronto, a city with generally lower levels of particulate and oxidant-gas pollution than Los Angeles, NO_2 showed a stronger association with hospital admissions than did particulate pollution or ozone (Burnett et al., 1999). Although NO_2 may be considered a traffic-related surrogate, it commonly coexists with PM and possibly exerts independent or additive effects.

We hypothesized from the evidence just described that combined exposure to CAP ($\sim 200 \ \mu g/m^3$) and NO_2 (0.4 ppm), both at "worst-case" ambient levels, generates more marked effects than exposure to either pollutant alone. On the basis of epidemiologic evidence, we also hypothesized that subjects with COPD would be more susceptible than healthy elderly subjects, although that was not necessarily true in our recent study with CAP exposures alone (Gong et al., 2004). To test these hypotheses, we used the protocol for exposure and response measurements reported in our previous studies (Gong et al., 2003, 2004) to evaluate the separate and combined effects of CAP and NO_2.

METHODS

Previous publications describe in detail the design and operation of the exposure system and chamber, the experimental protocol, and the recruitment and screening of elderly subjects (Gong et al., 2000, 2003, 2004). In brief, the exposure apparatus consisted of a two-stage Harvard/EPA fine particle concentrator (Sioutas et al., 1997), interfaced to a single-person exposure chamber. Air delivered from the concentrator to the chamber passed through a high-efficiency particle (HEPA) filter during filtered air (FA) or NO_2 exposures. Ambient pollutant gases were not actively removed by the filter, but their eventual in-chamber concentrations were low in comparison to levels likely to elicit respiratory effects (as discussed in a later section). For CAP exposures, a varying portion of air from the concentrator passed through the HEPA filter to adjust the CAP concentration in the chamber, which was monitored in real-time using a Data-RAM nephelometer (MIE, Inc., Bedford, MA). Actual CAP exposure levels were determined retrospectively using a multistage micro-orifice uniform deposit impactor (MOUDI), which collected integrated samples during exposure studies for subsequent gravimetric and chemical analysis. Details about the chemical analyses performed on CAPs are described in our recent paper (Gong et al., 2004). Nitrogen dioxide was introduced into the chamber inlet from a pressurized cylinder (1% NO_2 in nitrogen, Air Liquide, Long Beach, CA) via a metering valve and was continuously monitored in real time using a commercial chemiluminescent analyzer (model 200A, Advanced Pollution Instrumentation, Inc., San Diego, CA). Instrument performance was verified by pre- and poststudy calibration, using the appropriate calibration source gases and/or factory calibration protocols.

The experimental protocol and consent form were reviewed and approved by the local Institutional Review Board. All volunteers were recruited via invitation of eligible previous volunteers and by media advertisements. Each volunteer gave written informed consent prior to screening procedures, which consisted of medical history, routine physical examination, lung function testing, and submaximal exercise. The two groups of elderly subjects (Table 1) were reasonably matched except for

TABLE 1
Subject characteristics

	Healthy ($n = 6$)	COPD ($n = 18$)
Gender	4 F, 2 M	9 F, 9 M
Ethnic group	5 White	17 White
	1 Hispanic	1 Hispanic
Age, yr	68 (11)	72 (7)
Height, cm	161 (10)	168 (12)
Weight, kg	80 (12)	76 (22)
FEV_1, % predicted[a]	106 (15)	54 (12)

Note. Values given as mean (standard deviation) or count.
[a] Morris et al. (1971).

their numbers, actual and predicted lung function (Morris et al., 1971), and health status. More COPD than healthy subjects were evaluated, for two reasons. First, COPD subjects were expected to be more susceptible, so that their responses would be more important to document for purposes of health risk assessment. (Healthy subjects were studied initially, primarily to verify the safety of exposing others at higher risk.) Second, COPD subjects' responses were expected to show high variance, so that a relatively large sample would be required to maximize statistical power. In retrospect, a larger proportion of normal subjects would have been desirable, because they showed more evidence of response (see Results). Subjects with the diagnosis of COPD were former long-time smokers (>20 pack-years), who had quit smoking for >1 yr, with current chronic cough and/or breathlessness and moderate to severe airflow obstruction (American Thoracic Society, 1995; NHLBI/WHO, 2001), without evidence of clinically significant airflow reversibility or active cardiovascular disease. Patients with prescribed bronchodilators and corticosteroids were allowed to continue their use during the study, provided that they maintained a consistent dosage in proximity to each experimental exposure. No subject was taking inhaled or systemic corticosteroids, leukotriene modifiers, or supplemental oxygen. The healthy subjects were asymptomatic with normal baseline lung function and no history of significant smoking or evidence of clinically significant cardiovascular disease.

The experimental design required that each subject rest in a clean-air room in the laboratory for about 1 h prior to exposure. He/she was then exposed on separate days to one of the following four conditions, each separated by at least 2 wk: (1) filtered air (FA); (2) 0.4 ppm NO₂; (3) concentrated ambient particles (CAP), predominantly in the fine (PM₂.₅) size range, at concentrations near 200 $\mu g/m^3$; and (4) CAP and NO₂ together. Due to logistical constraints, CAP and FA exposures were initially conducted (in randomized order) in 4 healthy and 12 COPD subjects, followed by NO₂-containing exposures (in randomized order) 2–3 mo later. As a result, exposure order was not fully counterbalanced with respect to NO₂ exposures, which more often occurred at later dates and/or in warm seasons than

exposures without NO₂. Each exposure lasted 2 h (with unencumbered breathing), encompassing four 15-min periods of mild exercise (doubling minute ventilation) interspersed with four 15-min periods of rest. The subject's electrocardiogram and arterial oxygen saturation (SpO₂) via pulse oximetry (model N-3000, Nellcor, Pleasanton, CA) were monitored continuously during each exposure and for 4 h afterward, during which time the subject rested in filtered air. Spirometric measurements, including forced vital capacity (FVC), forced expired volume in 1 s (FEV_1), and maximal mid-expiratory flow (MMEF), were performed shortly before exposure began ("pre"), immediately after exposure ended ("post"), at the end of the 4-h postexposure rest period ("4 h"), and at a return visit the next day ("day 2"), about 22 h after the end of exposure. Symptoms were recorded on a questionnaire (with a standardized grading system) every 15 min during exposure and at the time of each lung function measurement, when blood pressure (by Sunbeam model 7657 automated monitor, calibrated against a mercury-column sphygmomanometer), heart rate, and SpO₂ were also recorded. Minute ventilation was measured using a portable respirometer for about 1.5 min during the last 5 min of the second and fourth rest and exercise periods of exposure. During the day 2 visit, spirometry, sputum induction (Fahy et al., 1996) with 3% saline, and total sputum cell counts were performed. Sputum cell differential was subsequently counted (Gong et al., 2003).

Statistical analyses were performed with BMDP software (1993 edition, SPSS, Inc., Chicago). Because some subjects did not undergo all four exposures (see Results), analysis of variance (ANOVA) with repeated measures on subjects and maximum-likelihood estimation of missing data (program 5V) was used as the basic analytical tool. Factors of the analytical models were clinical group (healthy vs. COPD), time of measurement (usually pre, post, 4 h, day 2), CAP (present vs. absent), and NO₂ (present vs. absent). A time effect could not be determined for sputum measurements, which were taken only on day 2. Main effects and interactive effects of these factors were estimated; an effect was considered significant at $p < .05$. If a main or interactive effect of CAP or NO₂ on a given response variable was significant in the initial "all-inclusive" analysis, further analyses were performed to determine the significance of before-to-after-exposure changes in that variable (see Results). In addition, linear regression analyses were performed to test whether individual responses varied in relation to individual exposure levels of CAP or its components, and whether baseline health measures varied in relation to ambient pollution levels in the vicinity of the laboratory, measured over 24-h periods preceding exposures.

RESULTS

Exposures

All 6 healthy subjects completed all exposures, as did 16 COPD subjects. One COPD subject withdrew after completing three exposure studies, and another after completing two

TABLE 2
Exposure measurements

Measure	FA	NO_2	CAP	$CAP + NO_2$
Total particles	22 (12)	21 (12)	189 (28)	203 (51)
Ultrafine ($<0.1\mu$m)	6 (6)	5 (3)	7 (4)	6 (4)
Fine (1–2.5 μm)	10 (5)	10 (5)	164 (26)	178 (47)
Coarse (>2.5 μm)	6 (5)	6 (5)	18 (7)	18 (8)
NO_3^-	NM	NM	48 (18)	50 (29)
SO_4^{2-}	NM	NM	20 (14)	29 (19)
Silicon	NM	NM	4.0 (1.6)	3.9 (2.3)
Iron	NM	NM	2.9 (1.4)	2.6 (1.5)
Elemental carbon	0.7 (0.9)	0.3 (0.4)	10.1 (6.6)	9.3 (11.8)
Aluminum	NM	NM	1.6 (0.8)	1.7 (1.2)
Calcium	NM	NM	2.3 (0.9)	2.2 (1.0)
Sodium	NM	NM	2.0 (2.1)	1.9 (2.2)
Potassium	NM	NM	1.1 (0.5)	1.1 (0.4)
Chlorine	NM	NM	2.5 (1.6)	2.7 (2.3)
NO_2 (ppb)	32 (30)	402 (9)	42 (21)	399 (10)
Temperature ($^\circ$C)	24 (2)	25 (1)	24 (2)	25 (1)
Relative humidity (%)	58 (12)	62 (10)	59 (9)	60 (13)
Ambient PM_{10} (past 24 h)	29 (12)	31 (10)	37 (8)	35 (9)

Note. Data are mean (standard deviation). NM, not measured. Units are μg/m^3 unless otherwise indicated. Exposures of healthy and COPD subjects were not significantly different except for ambient PM_{10} (see text).

studies; their data were retained for analysis. Four other volunteers who withdrew after only one exposure were excluded from all data analyses. The reasons for withdrawal were unrelated to the exposures. The target concentrations for fine PM and NO_2 were reasonably well achieved inside the chamber during exposures (Table 2). Total particulate mass concentrations in CAP and CAP + NO_2 exposures averaged close to the target of 200 μg/m^3, although variation between individual studies was appreciable. Ultrafine (<0.1 μm) particles contributed on average about 6 μg/m^3 under all exposure conditions, since ultrafine PM was neither effectively concentrated nor removed from filtered air. Coarse (>2.5 μm) particles contributed on average about 6 μg/m^3 in FA or NO_2 exposures, reflecting particles generated inside the chamber by subjects and/or exercise equipment, and about an additional 12 μg/m^3 in CAP or CAP + NO_2 exposures, reflecting modest enrichment by the concentrator of ambient particles larger than 2.5 μm. In CAP and CAP + NO_2 exposures, fine (0.1–2.5 μm) particle concentrations averaged near 170 μg/m^3 or >85% of total mass concentration. In FA and NO_2 exposures, fine particles averaged near 10 μg/m^3, reflecting in-chamber particle generation. Given that in-chamber generation should have contributed a similar amount during CAP and CAP + NO_2 exposures, the mean exposure level of actual concentrated ambient fine particles was near 160 μg/m^3. Particle composition was similar to that measured in a previous study at the same site (Gong et al., 2003). Nitrate, sulfate, organic and elemental carbon, and crustal elements were important components. Although measured, organic carbon concen-

trations could not be quantitated accurately because of positive measurement artifacts, as observed in our previous study (Gong et al., 2004), and thus are not reported in Table 2. Pairwise correlations and principal-components analyses were performed to explore relationships among different particulate components in exposures including CAP. Total mass concentrations correlated fairly strongly with nitrate ($r = .6$ for CAP alone, .7 for CAP + NO_2 exposures), but weakly with elemental carbon or crustal elements ($r = .2$ to .4), and not at all with sulfate ($r < .05$). Principal-components analysis of data for the eight most abundant species in elemental analysis identified three factors. The first represented crustal elements—aluminum, silicon, calcium, potassium, and iron—of which silicon was most abundant, followed by iron. These five species accounted for, on average, about 20 μg/m^3 of the particulate mass, assuming they existed as oxidized compounds. The second factor represented sulfur, elemental concentrations of which were consistent with ion-chromatographically determined concentrations of sulfate. The third factor represented sodium, which was uncorrelated with other elements, but accounted for only 2 μg/m^3 of the particulate mass, on average. In light of these results, total mass, sulfate, elemental carbon, and silicon were selected as useful variables for exposure-response analyses (see later description).

Generated concentrations of NO_2 were consistently close to the target of 0.4 ppm (400 ppb). In FA and CAP exposures, NO_2 concentrations were close to concurrent ambient levels, averaging near 40 ppb. Ambient PM_{10} concentrations during preceding 24-h periods, averaged from 2 monitoring stations

nearest the laboratory, were significantly lower for exposure studies without CAP than for those with CAP (means of 30 and 36 μg/m^3, respectively; CAP main effect $p = .02$). This difference is attributable to the rescheduling of CAP exposures away from days with unusually low ambient pollution that would have reduced the likelihood of reaching the target particulate concentration. Prior-24-h ambient NO₂ concentrations from the same 2 monitoring stations averaged 38 ppb overall, and showed no significant variation with experimental exposure conditions. Chamber temperature averaged about 1°C higher in exposures with NO₂ than without ($p < .05$). Relative humidity was not significantly different, but also averaged higher with NO₂ than without. These differences reflect the previously mentioned delay in some NO₂-containing exposures.

Responses: Physiology

Mean forced expiratory lung function measurements for healthy and COPD subjects did not change to a clinically meaningful extent in group or individual results with any exposure

MEAN SPIROMETRIC MEASUREMENTS - HEALTHY

(a)

MEAN SPIROMETRIC MEASUREMENTS - COPD

(b)

FIG. 1. (a) Mean FVC, FEV1, and MMEF for healthy subjects before and after the four exposures. (b) Mean FVC, FEV1, and MMEF for COPD subjects before and after the four exposures.

(Figure 1). Changes in FVC and FEV₁ during/after exposure, relative to preexposure values, were nonsignificant. Most atmosphere effects detected by ANOVA were main effects that were evident both before and after exposure, attributable to incompletely controlled extraneous factors rather than to the experimental atmospheres. However, MMEF showed unfavorable changes in healthy subjects only (Figure 2); before-to-after-exposure changes showed a significant (ANOVA, $p < .01$) negative effect of CAP—that is, MMEF decreased more after CAP or CAP + NO₂ exposures than after FA or NO₂ alone. The MMEF reduction was delayed in both CAP conditions (although by varying degrees) and was greater with CAP alone.

As expected, COPD subjects had a lower mean SpO₂ than healthy subjects ($p = .01$ for group difference) (Figure 3). The before-to-after-exposure changes of SpO₂ showed a significant ($p < .05$) overall acute negative effect of exposures to CAP
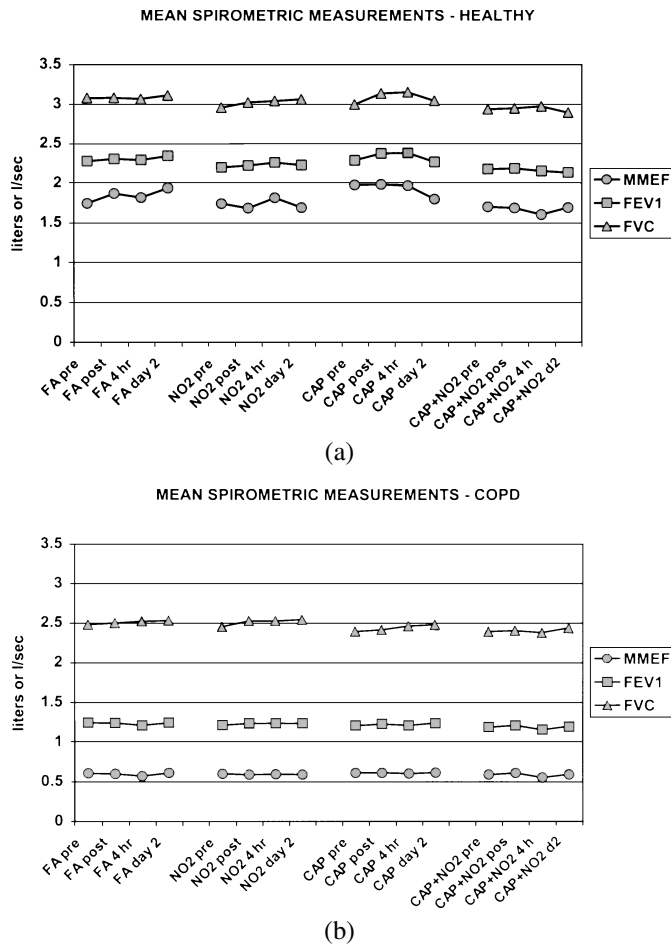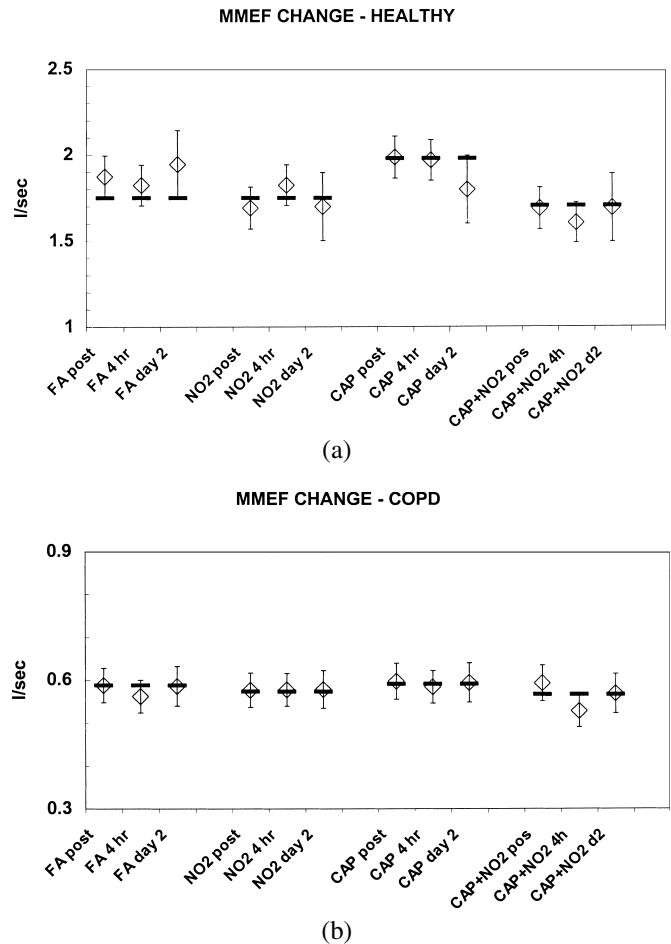
MMEF CHANGE - HEALTHY

(a)

MMEF CHANGE - COPD

(b)

FIG. 2. (a) Maximal mid-expiratory flow (MMEF) in healthy subjects under the four exposure conditions. Horizontal bar = preexposure mean. Diamond = postexposure mean. Error bar = 95% confidence interval of change from preexposure value. (b) Maximal mid-expiratory flow (MMEF) in COPD subjects, plotted in the manner of (a).
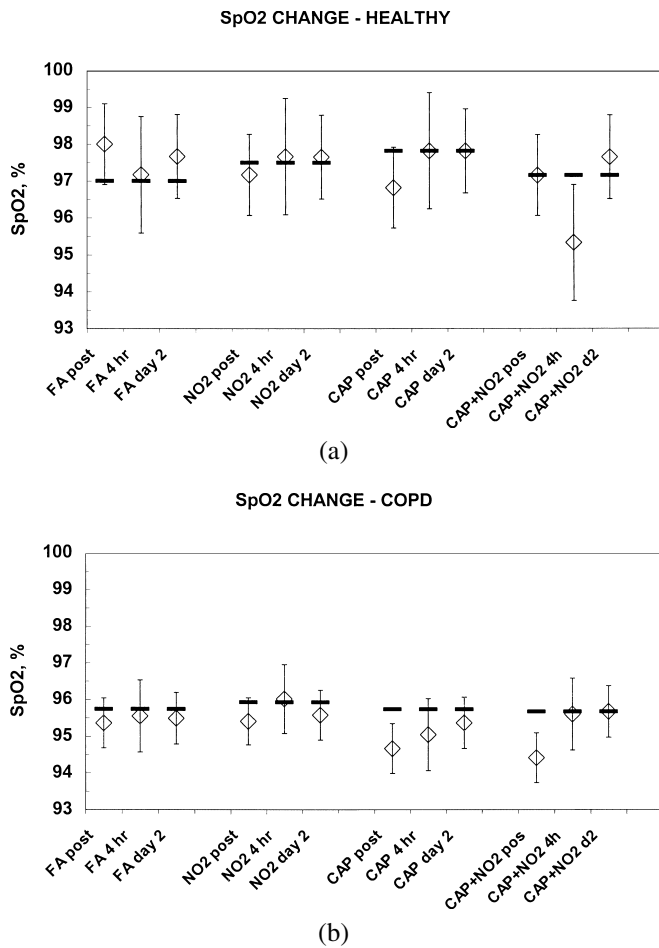
SpO2 CHANGE - HEALTHY

(a)

SpO2 CHANGE - COPD

(b)

FIG. 3. (a) Arterial oxygen saturation (SpO_2) as measured by pulse oximetry in healthy subjects under the four exposure conditions. Horizontal bar = preexposure mean. Diamond = postexposure mean. Error bar = 95% confidence interval of change from preexposure value. (b) Arterial oxygen saturation (SpO_2) in COPD subjects, plotted in the manner of (a).

alone and CAP + NO_2. The mean SpO_2 decrease attributable to CAP, averaged across all 3 times of measurement after exposure, was estimated as 0.7% for healthy and 0.3% for COPD subjects.

Overall mean systolic/diastolic blood pressure was 140/78 mm Hg. Blood pressure did not vary significantly by time, exposure atmosphere, or clinical status, except that diastolic pressure showed a slight decline at 4 h and day 2, relative to pre- and postexposure (main effect of time, $p < .05$). Concurrent heart-rate measurements showed a borderline-significant ($p \cong .05$) interactive effect of CAP and NO_2, not significantly different between healthy and COPD subjects. The overall mean rate before exposures was 74 beats/min. The increase after exposure (averaging all 3 times of measurement) was 3.1 beats/min with NO_2, 3.6 with CAP, 2.6 with CAP + NO_2, and 1.0 with FA. Thus, either pollutant alone seemed to increase heart rate slightly, while both pollutants' combined effects were indifferent or antagonistic. Minute volumes measured during exposure averaged 10 L/min at rest and 26 L/min during exercise in healthy subjects, 11 and 22 L/min, respectively, in COPD subjects. There was no significant variation by atmosphere.

Responses: Symptoms

The mean total preexposure symptom score was 2 in healthy subjects (reflecting one mild or two minimal symptoms) and 11 in COPD subjects (reflecting multiple clinically significant symptoms) ($p < .05$ for group difference). Neither the total score nor the respiratory, cardiac, or nonspecific symptom subtotals varied significantly by exposure condition. The total score increased by <1 point on average during and immediately after exposure, indicating a barely perceptible increase in one symptom in a typical subject; it subsequently fell below the preexposure level ($p < .01$ for main effect of time). Changes during and after exposure did not vary significantly by exposure atmosphere or by clinical status.
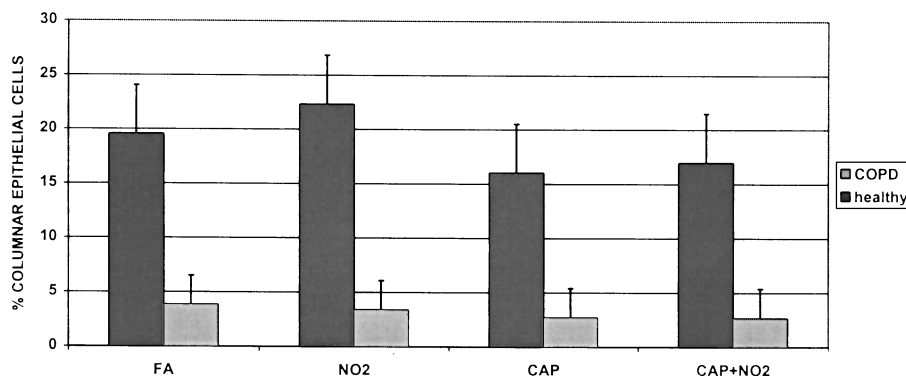

FIG. 4. Percentage of columnar epithelial cells in sputum induced 22 h after end of exposure (day 2). Bar = mean. Error bar = standard error of the mean.

Responses: Induced Sputum

Counts of total white blood cells and columnar epithelial cells in sputum showed no significant variation by exposure atmosphere. In differential cell counts, the percentage of columnar epithelial cells was much lower overall in COPD than in healthy subjects, but both groups decreased after exposures containing CAP (Figure 4). This negative effect of CAP was significant overall ($p < .01$ for main effect), and larger in healthy than COPD subjects ($p < .05$ for CAP–COPD interaction). No other cell type varied significantly by atmosphere. Table 3 summarizes these results.

Concentration-Response Relationships in Exposures

Regression analyses were performed to relate individuals' pre- to postexposure changes in FVC, FEV₁, MMEF, SpO₂, total symptom score, and heart rate with their measured exposure levels of total particle mass, sulfate, silicon, iron, and elemental carbon in CAP exposures. This analysis was done for COPD subjects separately and for all subjects pooled. (Healthy subjects were too few in number for meaningful separate analysis.) The trends of FEV₁ or FVC change versus sulfate (illustrated in Figures 5 and 6, respectively) were negative but nonsignificant in exposures to CAP alone. However, in the CAP + NO₂ exposures, the reductions were significantly ($p < .05$) related to sulfate levels, with regression slopes predicting losses >100 ml in FEV₁ and >200 ml in FVC at the highest observed sulfate exposure levels, relative to the lowest. These negative relationships remained significant when the analyses were limited to COPD subjects. Also, for COPD subjects only, the change in heart rate pre- to postexposure varied negatively with mass concentration in CAP exposures [slope −0.146 (beats/min)/(μg/m³), $p = .04$]. In CAP + NO₂ exposures, the corresponding slope was −0.08, not significant. As described earlier, heart rates in-
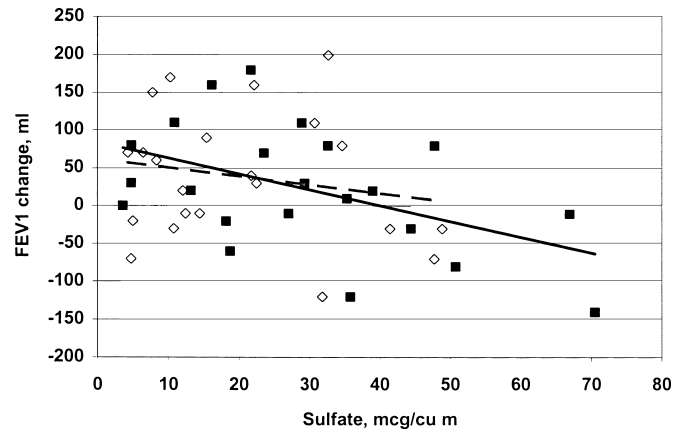

FIG. 5. Individual pre- to postexposure FEV₁ changes versus individual sulfate exposure concentrations. Solid squares and solid regression line = CAP + NO₂ exposures (relationship significant, $p < .05$). Open diamonds and dashed regression line = CAP exposures (relationship not significant).

creased after exposures, on average. Thus, the regression results indicate that the tendency to increase was less in the COPD subjects who were more heavily exposed. For all subjects, exposure concentrations of iron in CAP + NO₂ exposures showed a weak but significant negative relationship to change in total symptom score: Symptoms increased less during exposure when iron concentrations were higher.

Effects of Preexposure Ambient Pollution

Analyses of covariance were performed to relate prior 24-h average ambient PM₁₀ or prior 24-h average ambient NO₂ with preexposure lung function, total symptom score, systolic and diastolic blood pressure, heart rate, and SpO₂. Only the

TABLE 3
Sputum differential counts: Mean percentage of each cell type

Cell type	FA	NO₂	CAP	CAP + NO₂
Healthy subjects				
Columnar epithelials	19.5	22.3	16.0	17.0
Monocytes	15.0	21.6	26.3	19.0
Neutrophils	65.2	55.9	57.3	63.6
Eosinophils	0.2	0.2	0.4	0.4
COPD subjects				
Columnar epithelials	3.8	3.4	2.7	2.7
Monocytes	13.7	15.5	11.5	13.0
Neutrophils	80.7	79.6	84.1	82.2
Eosinophils	1.8	1.5	1.7	2.0

Note. Percentages may not add to 100 due to rounding and to maximum likelihood estimation of missing data in ANOVA. Only columnar epithelial cells showed a significant effect of exposure atmosphere; see text for explanation.
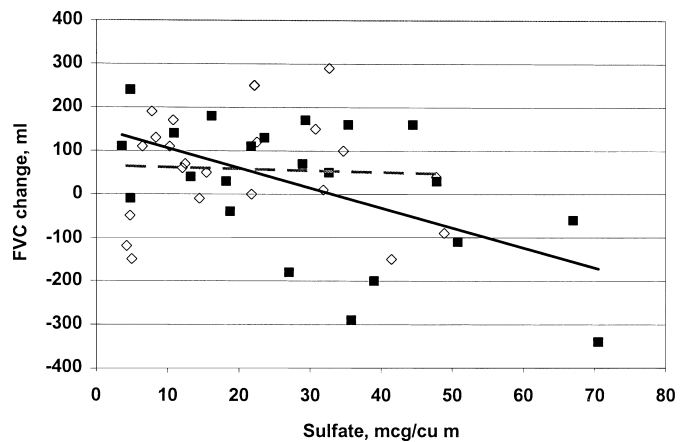

FIG. 6. Individual pre- to postexposure FVC changes versus individual sulfate exposure concentrations. Solid squares and solid regression line = CAP + NO₂ exposures (relationship significant, $p < .05$). Open diamonds and dashed regression line = CAP exposures (relationship not significant).

lung-function measures showed significance. For MMEF, the relationship to ambient PM_{10} or NO_2 was positive in healthy subjects (i.e., this function appeared to improve with increasing pollution) but near zero in COPD subjects (group by pollutant interaction, $p < .005$). For FEV_1 and FVC, the pattern was similar, but estimated slopes for COPD subjects were negative, and the FVC/PM_{10} relationship did not reach statistical significance. In analyses limited to COPD subjects, FVC and FEV_1 (but not MMEF) showed significant ($p < .05$) negative relationships to ambient NO_2. The estimated slopes were near -0.2%/ppb, predicting a decrement of roughly 10% in preexposure FVC or FEV_1 at the highest observed ambient NO_2 pollution levels, compared to the lowest.

DISCUSSION

Previous ambient fine-particle exposure studies of healthy and asthmatic adults here and elsewhere have yielded relatively little clinical evidence of acute respiratory responses. A relatively small respiratory response was also found in the present study, despite the inclusion of additional risk factors such as increased host susceptibility (advanced age and COPD) and the presence of a pollutant mixture. Neither the healthy nor the COPD group experienced measurable large-airway dysfunction (as measured by FVC and FEV_1), proximal airway inflammation (as measured by sputum cells), or increased respiratory or other symptoms during or after exposure to CAP, with or without NO_2. On the other hand, the findings of decreased MMEF and decreased SpO_2 are consistent with dysfunction in small peripheral airways resulting from exposures containing CAP. Both effects were more evident in healthy than in COPD subjects, supporting our previous findings of similar oxygen desaturation in healthy younger adults (mean age 50 yr) exposed to a more potent oxidant, ozone (Gong et al., 1998), and in the elderly (mean age $\sim$70 yr) with and without COPD exposed to fine PM (Gong et al., 2004). Possible explanations for the observed excess pulmonary effects (increased susceptibility?) in the healthy subjects could include a small number of subjects, allowing a low statistical power and a possible false-positive finding; a greater proportion of female subjects, with "smaller" lungs relative to body size than male counterparts; increased preexposure to ambient pollutants; chamber exposure to greater mass or chemical constituents than in the COPD subjects; and greater exercise during exposure. However, most of these possibilities appear unlikely. In general, the measured chamber exposure concentrations and other chamber conditions were not significantly different between healthy and COPD groups. One exception was prior 24-h average PM_{10} levels at nearby stationary monitoring stations that indicated ambient PM_{10} was higher in COPD than healthy subjects prior to CAP exposures, but higher in healthy subjects prior to exposures without CAP (clinical status $\times$ CAP interaction, $p = .03$). The differences were mild (20% or less, on average), so it seems unlikely that this observation confounded the significant results reported. On the other hand, COPD subjects may not have been able to change their

lung function (similar to the known partial airflow reversibility with acute bronchodilator administration in these patients). Residual bronchodilator effects may also have been on board to reduce airway changes in the COPD subjects, although the COPD subjects stated that they withheld their inhaled bronchodilators at least 8 h prior to exposure. These observations and previous findings of predominantly systemic rather than respiratory effects suggest that inhaled fine particles must penetrate and deposit in the deeper lung and thus affect the circulation to manifest their toxicity. Penetration of inhaled PM should be more effective through healthy airways, than through diseased airways, since relatively intact and greater distribution of ventilation is present in the former. More investigation of healthy elderly subjects exposed to CAP is warranted.

The CAP-associated decrease in percentage of columnar epithelial cells in sputum probably represents an effect on larger central airways, which are the major source of induced sputum (Alexis et al., 2001). We also found evidence for a decrease in columnar epithelial cells in younger healthy and asthmatic adults (Gong et al., 2003). The explanation for the apparent reduced shedding of these surface airway cells is unknown. This might reflect activation of a defense mechanism, such as a vagally mediated increase in airway mucus secretions causing removal of some columnar cells in the interval between exposure and sputum induction. Cholinergic stimulation may also elicit mild bronchoconstriction and increase peripheral airways resistance in both healthy and COPD subjects.

We found little support for our hypothesis of enhanced respiratory responses in combined exposures to CAP and NO_2. The only observed statistical interaction of CAP and NO_2 of even marginal significance was for a nonspecific response, that is, heart rate. Exposures to CAP or NO_2 alone provoked similar modest increases. The biological potency of a PM–gas combination may depend, in part, on the concentration and potency of the oxidizing gas, since low concentrations of ozone (0.1 ppm) increase the biological potency of diesel exhaust particles, while higher levels of ozone (1.0 ppm) decrease the biotoxicity (Madden et al., 2000). Combining PM and NO_2 (0.4 ppm) resulted in a slightly smaller (not larger) effect, although we did not evaluate a lower concentration of NO_2. The health significance of this phenomenon is uncertain. It might represent some antagonism between CAP and NO_2 effects with different interactive mechanisms, or a single generalized stress response to pollution exposure, indifferent to the type of pollutant. As far as is known, the generation of concentrated PM does not alter the particles' physical properties or potential for biological toxicity (Savage et al., 2003).

The observed association of higher sulfate exposure concentrations with more negative changes in FVC or FEV_1 after exposure is difficult to interpret, given the small subject sample and the nonsignificant overall CAP-related changes in FVC or FEV_1. Some epidemiologic findings point strongly to atmospheric sulfate as a likely cause of adverse effects, while others do not (e.g., Burnett et al., 2000; Gwynn et al., 2000; Harrison

& Yin, 2000; Brauer et al., 2001). Sulfate levels are generally lower in Los Angeles than in some northeastern or European cities where effects have been suggested. One likely explanation for the observed health outcomes may be that sulfate is a surrogate of other, potentially more toxic species, produced by photochemical reactions, which are also responsible for the production of sulfate (Fine et al., 2004; Stein & Lamb, 2003). More extensive CAP exposure studies in metropolitan Los Angeles during periods of intense photochemical activity may help to resolve this issue.

Other limitations of this clinical study include self-selected volunteers who are not necessarily representative of the vulnerable population from which they were derived; the small number of healthy volunteers, who unexpectedly showed stronger evidence of response than those with COPD; inability to directly measure peripheral-airway and alveolar effects; and somewhat artificial short-term exposure conditions with potential interference from exogenous factors, in particular, ambient pollution and weather conditions preceding experimental exposures. Another potential interference, only speculative at this time, is development of "tolerance" to ambient PM exposures during study participation. Additional clinical studies with larger elderly groups, as well as pooled analyses of data from multiple studies of concentrated fine particles, are warranted before drawing firm conclusions about the significance of the respiratory findings for public health and air quality regulatory policy. In particular, our results suggest that older adults with relatively healthy airways may be as or more vulnerable to particulate pollution than those with chronic airways disease. Thus, additional studies of healthy older adults should be a high priority.

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