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Concentrated Ambient Fine Particles and not Ozone Induce a Systemic Interleukin-6 Response in Humans

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Abstract

Epidemiological studies have established significant associations between ambient pollutants, including fine particulate matter ($PM_{2.5}$) and ozone (O_3), and cardiopulmonary morbidity and mortality. One mechanism that has been proposed is a pulmonary/systemic inflammatory response. Although controlled human exposure studies have examined the independent inflammatory responses of $PM_{2.5}$ and O_3, no studies have previously examined their joint effects. The study objective was to examine the independent and combined associations between ambient $PM_{2.5}$ and O_3 and acute respiratory/inflammatory responses. Using our concentrated ambient particle (CAP) facility for $PM_{2.5}$, we studied 10 mild asthmatic and 13 non-asthmatic individuals. The 2-hr exposures included CAP (range 48–199 $\mu g/m^3$) and filtered air (FA), with/without O_3 (120 ppb), in a randomized block design. Response measures included pulmonary function and inflammatory indices in induced sputum (IL-6, cytology) and blood (IL-6, TNF-α) measured before and after exposures. Three hrs post exposure there was an increase in blood levels of IL-6, but only after CAP alone exposures; the IL-6 increase was associated with increasing $PM_{2.5}$ mass concentration ($p=0.005$). Some individuals switched to shallow breathing during CAP+O_3, possibly accounting for an attenuation of the resultant blood IL-6 response. Asthmatic and non-asthmatic responses were similar. There were no adverse changes in pulmonary function or other inflammatory measures. The study demonstrated an acute IL-6 response to $PM_{2.5}$, providing

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DECLARATION OF INTEREST

The authors declare they have no competing financial interests.

evidence to support the epidemiological findings of associations between ambient levels of particles and cardiopulmonary morbidity and mortality.

INTRODUCTION

Epidemiological studies have demonstrated that increases in air pollutant levels are associated with adverse health effects (Burnett et al., 1998; Dockery and Pope, 1994; Dockery, 2001; Samet and Krewski, 2007). Both pulmonary and systemic effects have been reported. Two of the major components in urban smog, fine particles with an aerodynamic diameter less than 2.5 μm ($PM_{2.5}$) and ozone (O_3), are key contributors to these adverse health effects (Laden et al., 2006; Samet and Krewski, 2007; Schwartz et al., 1996). Although the mechanism of pollutant induced health effects is not clearly understood, it has been suggested that inhaled pollutants can initiate local airway inflammation, leading to a systemic inflammatory state (Brook et al., 2004). The process linking the initial pulmonary response to the subsequent or concomitant systemic inflammatory response has not yet been fully elucidated.

In epidemiological studies of the health effects of air pollution, mortality and hospital admissions have routinely been examined as health outcomes. Increasingly, there is also evidence of associations between ambient PM levels and intermediary inflammatory outcomes, such as white blood cells, interleukin (IL)-6, tumor necrosis factor alpha (TNF-α), C-reactive protein and fibrinogen (Delfino et al., 2008; Dubowsky et al., 2006; Rückerl et al., 2007; Samet and Krewski, 2007; Schwartz, 2001). A representative example is illustrated by a study of 30 young soldiers exposed to high levels of PM with an aerodynamic diameter less than 10 μm (PM_{10}, 125±7 $\mu g/m^3$) during a five-week long air pollution episode caused by forest fires in Southeast Asia. Systemic levels of IL-6, IL-1β and granulocyte-macrophage colony stimulating factor were increased over 75% during the forest fire “haze” compared to the post fire period when PM_{10} levels were reduced by 68% or 85 $\mu g/m^3$ (Tan et al., 2000; van Eeden et al., 2001). In a panel study of 44 non-smokers over 60 yrs of age, positive associations were observed between ambient levels of $PM_{2.5}$ (1–7 day moving average) and acute increases in IL-6, CRP and white blood cells (Dubowsky et al., 2006). In summary, epidemiological studies have shown positive associations between high as well as ambient levels of particles and acute health effects, in both healthy and susceptible populations.

Controlled human exposure studies have examined both respiratory and systemic acute effects of concentrated ambient particles (CAP) alone (Ghio et al., 2004), as well as the combined effects of CAP+O_3 on arterial diameter (Brook et al., 2002; Urch et al., 2004) and on blood pressure (Urch et al., 2005), but no previous study has compared the independent and combined effects of $PM_{2.5}$ and O_3 on inflammatory outcomes. The combined short-term effects of PM and O_3 have also been examined in time-series studies, with both mortality (Bell et al., 2004; Bell et al., 2005; Bell et al., 2007) and hospital admissions (Dominici et al., 2006) as health outcomes. The challenge of population studies is separating out individual pollutant effects from the pollution mixture (Sarnat et al., 2001; Sarnat et al., 2005). Controlled human exposure studies, on the other hand, provide a controlled exposure

atmosphere with the ability to compare acute responses to single or multiple pollutants and offer the opportunity to explore mechanisms.

We undertook a controlled human exposure study to examine and compare pulmonary and systemic inflammatory markers after 2-hr controlled exposures to Toronto fine CAP with or without O_3. Measures of response included pulmonary function, induced sputum cytology and the inflammatory cytokines IL-6 and TNF-α. Responses of mild asthmatics, a potentially more susceptible group, were compared to those of non-asthmatic individuals.

MATERIALS AND METHODS

Study Participants

Participants were recruited from the University of Toronto and the Greater Toronto Area. Controlled exposures were carried out between July, 1999 and February, 2003 at the Gage Occupational and Environmental Health Unit. The study was approved by the Human Subjects Research Ethics Review Committees of the University of Toronto and St. Michael's Hospital. Participants gave written informed consent prior to enrolment. Thirteen non-asthmatic and 10 mild asthmatic (11 male, 12 female) non-smokers of 18–40 years of age were enrolled. An initial screening visit ensured that participants met the inclusion criteria before they continued with the exposure phase of the study. Subjects were excluded if they had cardiovascular disease, diabetes or baseline spirometry <75% of the predicted normal values for forced vital capacity (FVC) and forced expiratory 1-sec volume (FEV_1) (Hankinson et al., 1999). All participants were free of respiratory tract infections for at least three weeks before exposure testing. Subjects underwent skin prick testing with 12 common inhalant allergen to test for atopy (Western Allergy Services, Canada). Asthmatics had physician-diagnosed asthma (subject-reported) that was confirmed by a positive methacholine challenge ($PC_{20} \leq 8$ mg/ml) and were untreated with inhaled or systemic corticosteroids for at least one month prior to and during their study participation. Short-acting inhaled bronchodilators were not taken after midnight prior to each visit. Non-asthmatics were excluded from the study if their PC_{20} was ≤ 8 mg/ml.

Exposure Groups

Participants were randomized to one of two groups: 1) no added O_3; or 2) added O_3 of 120 ppb (Table 1). Within each group, exposures were assigned in random order and included filtered air (FA) and target levels of both 60 and 150 $\mu g/m^3$ CAP. Not all subjects received the lower CAP exposure. After completing exposures within their O_3 group, subjects were asked if they were willing to carry on with exposures for the other O_3 group. Due to the lengthy time commitment involved, only eight subjects consented to continue, and completed exposures from both O_3 groups. There were six different exposure types and a total of 13–16 participants within each. Overall there were 88 exposures, 43 without and 45 with O_3. Exposures were separated by at least two weeks, to minimize possible carryover effects from the previous exposure.

CAP and O_3 Exposures

Subjects arrived at the lab around 0900 hrs for each exposure, to reduce the impact of diurnal variation. CAP exposures were carried out using a Harvard sequential, high-flow (1,000 L/min) two-stage virtual impactor designed to concentrate ambient particles between 0.15 and 2.5 μm aerodynamic diameter (Sioutas et al., 1997). The CAP facility also included a clean air/inlet dilution system to maintain CAP levels at the target concentrations, and a participant enclosure with face mask delivery. The CAP facility and exposure characterization have been described in detail previously (Petrovic et al., 2000; Urch et al., 2004; Urch et al., 2005). Briefly, the 2-hr average CAP and O_3 levels were determined using the gravimetric exposure $PM_{2.5}$ mass concentrations and the 15-sec O_3 analyzer concentrations (Dasibi, model 1008RS, Dasibi Environmental Corp., Glendale, CA). During exposures a sample was collected on a 47 mm Gelman Teflon 2 μm pore size filter. After exposures, temperature/humidity conditioned filters were analyzed for total mass followed by analysis of the major inorganic ions by ion chromatography (Dionex, model DX-300, Dionex Corp., Sunnyvale, CA).

Exposure Protocol

For each exposure a 3-day testing protocol was followed, as detailed with timelines for each test in Table 2. The exposure was carried out on day-2, with tests before (pre) and after (post). The following three sections describe the pulmonary function measures and exposure breathing parameters, induced sputum and blood cytokines.

Pulmonary Function and Exposure Breathing Parameters

Standardized testing procedures were followed (ATS, 1995) using equipment/methodology described previously (Liu et al., 1997). Measures included spirometry, lung volumes, airway resistance, and a single-breath diffusion test of carbon monoxide uptake. A methacholine challenge was carried out in asthmatics only, to determine the PC_{20}, the day before and after exposures. Respiratory minute ventilation (MV, L/min) and frequency (f_r, breaths/min) were measured at the start of the exposure and every 30 min thereafter with a precision turbine-type flow meter (VMM-401, Interface Assoc., Aliso Viejo, CA). The tidal volume (V_T, L/breath) was calculated as the ratio of MV to f_r.

Induced Sputum Cytology and Cytokines

Sputum was induced through inhalation of hypertonic saline (3, 4, then 5%) nebulized with an ultrasonic nebulizer (Liu et al., 1999). Sputum plugs were extracted and processed as per Pizzichini et al. (1996). A technician blinded to the exposure type performed differential cell counts by identifying 400 cells as neutrophils, macrophages, eosinophils, lymphocytes, basophils or bronchial epithelial cells. Two non-asthmatics and one asthmatic were unable to produce sputum samples. In only half of the 88 exposures were subjects able to produce sputum samples for pre and post exposures. Sputum supernatants were analyzed for IL-6, IL-8, IL-10, TNF-α and leukotriene-B_4 using ELISA kits (Amersham Pharmacia Biotech, NJ). Some sputum samples yielded low supernatant volumes, insufficient for cytokine analyses, which resulted in IL-6 data available for only 30/88 (34%) of exposures (pre and

post) and less for the other mediators. Since data was even less sparse within each of the six exposure types, we do not report results for any sputum mediators.

Blood IL-6 and TNF-α

Venous blood was collected in EDTA tubes and plasma frozen at −70°C. The plasma was later analyzed for IL-6 and TNF-α using ELISA kits.

Statistical Analysis

The study design was a randomized block with subjects randomly assigned to no O_3 or added O_3 groups (see Exposure Groups). Thus, each person acted as their own control. In order to examine the exposure-induced change, the post minus pre-exposure difference (Δ) was calculated for each response variable, separately for the three post exposure time points (10 min, 3 and 20 hrs). For breathing parameters measured during exposure (e.g., tidal volume) and pulmonary function, the percent change (%Δ) was calculated. Analysis of variance and multiple regression was used to assess the association between these measures of change (Δ, %Δ) and CAP, O_3 and their interaction using the SAS MIXED procedure (SAS Institute, Inc., Cary, NC, version 9).

We initially hypothesized that we would observe dose response associations between the 2-hr exposure mass concentrations and study outcome measures. We thus pre-selected exposure CAP target levels (60 and 150 μg/m^3) that we hoped would be distinct from each other. However, the CAP exposure level control was not as precise as that for O_3 levels, and when gravimetric PM determinations were completed there was some overlap in mass concentrations between the 60 and 150 μg/m^3 CAP groups. This overlap in mass concentrations would have made interpretation of the results difficult for inter-group comparisons when treating CAP as categorical. In order to obtain a distinct dichotomy of CAP levels, we redefined the two CAP groups as <100 and ≥100 μg/m^3, or roughly mid-point between the target levels of 60 and 150 μg/m^3. As a result, three individuals that had CAP targets of 60 μg/m^3, but in fact received higher gravimetric mass concentrations, were now in the ≥100 μg/m^3 CAP group, and one individual that had a CAP target of 150 μg/m^3, was now in the <100 μg/m^3 CAP group.

With the re-defined CAP groups, an initial analysis was carried out using O_3 as a binary variable and CAP as a 3-level categorical variable, considering the effects of CAP with a mass concentration <100 and ≥100 μg/m^3 compared to exposures without CAP (FA and FA +O_3). Each model included a random subject effect. In a second approach, CAP was considered as a continuous fixed effect and O_3 as a binary variable, since O_3 levels were relatively constant during O_3 exposures, whereas CAP levels varied during CAP exposures. We also examined the possible modification of CAP effects by O_3 (CAP-O_3 interaction) and by asthma status. In order to examine associations within a given exposure (e.g., for CAP +O_3 exposure, association between tidal volume 2-hr %Δ and 3-hr post IL-6 Δ), the Pearson correlation coefficient was calculated. Differences in physical characteristics, and baseline spirometry and mediators between asthmatics and non-asthmatics were tested using an unpaired t test. Data are reported as mean values ± standard errors.

RESULTS

Participant Characteristics

The 23 participants had a mean age of 27±1 years, ranging from 21 to 40, and normal spirometry— FEV_1 and FVC were >90% of the predicted normal values (Table 3). Asthmatics had a lower mean FEV_1/FVC ratio than non-asthmatics (79±2% vs. 85±2%, p=0.03) and higher sputum eosinophils (3.6±1.2% vs. 0.4±0.2%, p=0.03, data not shown). The methacholine PC_{20}, which defined the two groups, ranged from 0.08 to 7.6 mg/ml for asthmatics. Baseline blood IL-6 and TNF-α were not significantly different between asthmatics and non-asthmatics.

Exposure PM, Gaseous and Environmental Measurements

The mean $PM_{2.5}$ concentrations for $CAP_{\geq 100}$ and $CAP_{\geq 100}$+ O_3 were quite similar (140 and 142 μg/m^3, respectively) and approximately double those for the lower CAP groups (64 and 68 μg/m^3) (Table 4). The mean O_3 concentrations were close to the 120 ppb target (117–119 ppb) and showed less variability than $PM_{2.5}$. The other gaseous pollutant levels, including O_3 for the no O_3 group, were below ambient levels. For exposures with added O_3, nitric oxide (NO) levels were lower and nitrogen dioxide (NO_2) levels higher compared to the no O_3 group. This was an experimental artefact, as the ambient NO in the inlet air reacted with the added O_3 to form NO_2, thus increasing NO_2 and decreasing NO levels; even so, the sum of NO+NO_2 was still below the ambient NO+NO_2 level.

Exposure Breathing Parameters and Pulmonary Function

There were no significant effects of CAP or O_3 for the 2-hr percent change in exposure breathing parameters (MV, f_r, V_T) or for the percent change in any pulmonary function measures including the PC_{20} of the asthmatics (data not shown).

Sputum Cytology

No significant CAP- or O_3-induced changes were observed in sputum total cell counts. Furthermore, the two main cell types, macrophages and neutrophils, also showed no significant changes in the absolute number or percentage of cells (data not shown).

Blood IL-6

There were no significant effects of CAP or O_3 on Δ IL-6 levels immediately after or the day after the 2-hr chamber study in the 3-level CAP models. At 3 hrs after exposure, however, for CAP alone exposures with a mass concentration ≥100 μg/m^3, blood IL-6 levels were increased above pre-exposure; the least square mean increase was 0.29 pg/ml (95% CI: 0.16, 0.42, p<0.0001). The 3-hr post Δ IL-6 for CAP <100 μg/m^3, with or without O_3, did not increase, with responses similar to those of FA and FA+O_3. Since we only observed differences in Δ IL-6 between the two levels of CAP, we further examined the Δ IL-6 association for CAP-containing exposures only, treating CAP as a continuous variable (Figure 1). For CAP alone exposures we observed a significant positive association of mass concentration with the 3-hr post Δ IL-6 levels (slope= 0.00371 × mass, p=0.005), compared to no association for CAP+O_3 exposures (slope= −0.00034 × mass, p=0.76). Thus there was

significant modification of CAP effects by O_3 (CAP and O_3 interaction, p=0.021). Ozone, as a main effect, and having asthma (covariate) were not significantly associated with the 3-hr post Δ IL-6 (p=0.14 and 0.66, respectively).

Exposure Tidal Volume versus Blood IL-6

The latter finding of no increase in IL-6 after CAP+O_3 compared to CAP alone led us to examine whether the pattern of breathing in individuals differed between the CAP+O_3 and CAP exposures, potentially modifying the IL-6 responses. The result of this further investigation was, for CAP+O_3 exposures, we observed a significant positive association between the 2-hr percent change in V_T during exposure and the post exposure change in blood IL-6, at all three post exposure time points (Figures 2a–c). The correlation coefficients ranged from r=0.54 to 0.59 ($p \leq 0.01$). In stark contrast, for CAP exposures without O_3, there were no significant associations ($p > 0.3$) between V_T and IL-6 (Figures 2d–f). Respiratory MV was weakly positively associated with the CAP+O_3 IL-6 change (r=0.32 to 0.38, $p \geq 0.06$), while f_r was not associated with IL-6 ($p > 0.2$) (data not shown).

In Figure 1, it is apparent that IL-6 did not increase after CAP+O_3 exposures (slope= −0.00034 × mass), as well as after O_3 alone exposures (mean decrease of 0.06±0.06). Furthermore, for CAP+O_3 we observe that as V_T decreases so does IL-6 after exposure, but not after CAP alone. Thus, the presence or absence of O_3 with CAP may be an important factor. To better visualize this association, we created a 2×2 table of the binary 2-hr %Δ in V_T and the binary 3-hr post Δ IL-6, separately for O_3 and no O_3 exposures; both variables were dichotomized as a decrease, or an increase ≥0 (Table 5). Results for the 39 exposures with O_3 showed that 70% of the 20 responding with a decrease in V_T, had a corresponding decrease in IL-6, compared to only 32% among the 19 who responded with no decrease in V_T (p=0.026). No significant differences were seen for the corresponding changes among those not exposed to O_3.

Blood TNF-α

There were no significant CAP or O_3 induced changes in blood TNF-α at any of the post exposure time points.

DISCUSSION

In a controlled human exposure study, we demonstrated a $PM_{2.5}$-associated increase in systemic blood IL-6, but only for CAP exposures without added O_3. This finding was contrary to our *a priori* hypothesis of a more adverse response to CAP+O_3. We explored three possibilities to explain this. First, although the overall mean PM mass concentrations were similar for the CAP and CAP+O_3 exposure groups (107±8 versus 111±8 μg/m^3, p=0.72), we could not control individual PM constituent levels, which might have differed enough between CAP and CAP+O_3 exposures to explain the different responses. The study design was a randomized block, so this should have helped to reduce any constituent level exposure bias. A comparison of CAP versus CAP+O_3 constituent concentrations of ammonium, sulphate and nitrate showed similar levels across the $CAP_{<100}$ and $CAP_{\geq 100}$ exposures (Table 4). We did not measure elemental carbon, organic carbon, or trace

elemental constituents of CAP. It is possible that they differed between CAP and CAP+O_3 exposures, but unlikely given the randomized sequence and the similarity of other constituents. Thus, differences in CAP with and without ozone are unlikely to explain differences in response. Second, 15/23 subjects were different between the CAP and CAP +O_3 exposure groups. We thus examined group differences that could have accounted for the heterogeneity in blood IL-6 responses. Baseline characteristics and other exposure measures such as minute ventilation and pulmonary function changes revealed no significant differences between the two groups. However, individuals exposed to CAP alone had a significantly lower pre-exposure IL-6 compared to those individuals exposed to CAP+O_3 (0.46±0.07 vs. 0.81±0.14 pg/ml, p=0.04); yet for CAP-containing exposures the pre-exposure IL-6 was not significantly correlated with the 3-hr post Δ IL-6 (r= −0.19, p=0.18). Thus, differences among individual subjects are unlikely to explain differences in IL-6 response by the CAP and CAP+O_3 groups.

A third possible explanation is that breathing patterns differed between CAP and CAP+O_3 exposures, causing differences in inhaled particle deposition and the resulting IL-6 response. Indeed, for CAP+O_3 exposures we observed a significant positive association between the 2-hr tidal volume percent change and the post exposure IL-6 change, at all three time points (Figures 2a–c). However, in dramatic contrast, we saw no associations of any breathing parameters with IL-6 for CAP alone exposures (Figures 2d–f). Thus, for CAP+O_3 exposures, we can infer that individuals who had a decrease in their tidal volume over the course of the exposure had a corresponding decrease in IL-6 after. The plausibility of this inference is strengthened by the fact that all three time points showed a similar strength of the association. Of note, among the O_3 exposures 70% of the individuals who exhibited shallow breathing had a decrease in IL-6, compared to only 32% among those without shallow breathing (p=0.026). This difference was attenuated for exposures without O_3, 41% versus 24%, (p=0.32). This weakly suggests that O_3 may have been the stimulus of this response.

The latter finding of breathing pattern changes during exposures provides insight into the mechanism of PM-induced inflammation, specifically when PM is combined with O_3 as an ambient co-pollutant mixture. The shallow breathing response that we observed has been reported previously for O_3 exposures (2 hrs at 500 ppb, with intermittent exercise) by Hazucha et al. (1989). The mechanism the authors proposed was reflex inhibition of respiratory effort, likely originating from airway neural C-fibers. The effects of different breathing patterns on airway particle deposition (Finlay and Martin, 2008; Heyder et al., 1975; Kim and Hu, 2006) and O_3 uptake (Ultman et al., 2004) have been examined, showing an association between lower tidal volumes and less PM deposition/O_3 uptake. Our finding that some individuals switch to shallow breathing during CAP+O_3 exposures and that this in turn may attenuate the systemic IL-6 response, suggests a protective mechanism. It was not apparent that there were any exposure cues to identify the CAP+O_3 exposures. When subjects were asked if they thought they had been exposed to a pollutant, only 52% (15/29) of the CAP+O_3 exposures were correctly identified. Furthermore, there was no significant difference in tidal volume changes between those who correctly identified the pollutant exposures vs. those who didn’t (data not shown). The latter evidence supports the hypothesis that the altered breathing pattern was most likely an involuntary reflex, possibly mediated

through airway C-fiber stimulation by O_3, or a combined effect of particles and O_3. An irritant reflex mechanism has been proposed as a response to inhalation of O_3 (Hazucha et al., 1989; Lee and Widdicombe, 2001) and particles (Passannante et al., 1998).

In other human CAP studies, Ghio et al. (2003) reported no significant changes in blood TNF-α or IL-6 immediately and 20 hrs after 2-hr exposures with intermittent exercise (IE) to fine CAP (121±14 μg/m^3) compared to FA. Gong et al. (2003) observed a 36% increase in blood IL-6 among asthmatics 4 hrs after a 2-hr exposure with IE to fine CAP (174±8 μg/m^3), which was similar to our mean 56% increase in IL-6 after $CAP_{\geq 100}$ (59% for asthmatics, 55% for non-asthmatics). Gong's reported 18% increase in IL-6 for asthmatics after FA was similar to our observed increase of 8%. In contrast though, Gong and authors observed a 26% increase in blood IL-6 among non-asthmatics and this response was not greater than that of FA (39% increase). Similar to Ghio et al. (2003), we also did not observe any significant CAP-induced changes in TNF-α. Delfino et al. (2008) measured blood levels of mediators including CRP, IL-6, TNF-α and the TNF-α soluble receptor II in a panel of 29 non-smokers over 65 yrs of age that had a history of coronary artery disease. The authors showed significant positive associations between markers of primary combustion products ($PM_{2.5}$ elemental and black carbon and primary organic carbon) and IL-6, CRP and TNF-α soluble receptor II. Associations were positive but largely non-significant with the cytokine TNF- α. They suggested that the differences in significance for TNF-α may have been due to the shorter half-lives and lower levels for TNF-α compared to its soluble receptor. Thus our finding of no significant changes in TNF-α may not necessarily indicate lack of response.

The association between CAP exposure and IL-6 response was not modified by having asthma. Although one might have expected a greater response in the individuals with asthma, the asthmatics who participated in our study were well-controlled and mild, as evidenced by a mean FEV_1/FVC ratio of 79% compared to 85% for non-asthmatics, and baseline sputum eosinophils of 3.6%, although significantly elevated when compared to the 0.4% for non-asthmatics, also indicative of very mild inflammation. The fact that our mild asthmatic subjects were not more susceptible should, however, not be generalized to represent responses for more severe asthma or for other respiratory diseases.

The strength of our study was that we compared CAP+O_3 responses to the individual pollutant responses, whereas previous studies have only examined CAP alone. The CAP target $PM_{2.5}$ concentration of 150 of μg/m^3 (maximum, 199 μg/m^3) is higher than that typically observed in urban environments over 24 hrs. However, levels exceeding 150 μg/m^3 can occur for one to two hr periods in many North American locations and are commonly encountered over even longer durations throughout developing nations (Michaels and Kleinman, 2000; Streets et al., 2007). In a panel study of asthmatic children living in Mexico City, high levels of daily PM_{10} > 200 μg/m^3 (~100 μg/m^3 $PM_{2.5}$) and hourly O_3 > 100 ppb were observed simultaneously about 25% of the time over an 8 month period (Romieu et al., 1996). Not all subjects received the six exposures due to the lengthy time commitment; even so, the dependent variables in statistical analyses were the change over time within a subject, thus each person acted as their own control. Many variables were measured: pulmonary function/ breathing parameters (15); sputum cytology (5); and two blood measurements

including TNF-α and IL-6, the latter being significant. No multiple comparison adjustment was applied because our lack of fine CAP-induced pulmonary function changes is supported by other studies (Ghio et al., 2000; Gong et al., 2003) and the sample size for the five sputum cytology variables was too small. Strengthening the plausibility of our main finding of an increase in IL-6 with CAP exposure was the significant association between IL-6 and exposure mass concentration.

In conclusion, for healthy individuals and mild asthmatics we have demonstrated a transient increase in systemic IL-6, 3 hrs after CAP exposure. Furthermore, the blood IL-6 increase was significantly associated with the $PM_{2.5}$ mass concentration and not O_3. We hypothesize that modification of the CAP response by concomitant O_3 exposure may be modulated through the pulmonary autonomic response to CAP+O_3 in some of our subjects, resulting in reduced tidal volume and reduced IL-6 response. Acute effects of this magnitude in healthy individuals are accommodated by normal defence mechanisms, thus are transient and sub-clinical. However, more prolonged exposures at elevated PM levels and episodic exposures in susceptible populations provide an increased risk. Our data provide evidence to support the association between ambient levels of particles and cardiopulmonary morbidity and mortality. We did not show a greater effect with the concomitant CAP+O_3 exposure, but did provide some insight that may help to explain equivocal epidemiological findings when comparing health effects of PM and O_3, alone and combined.

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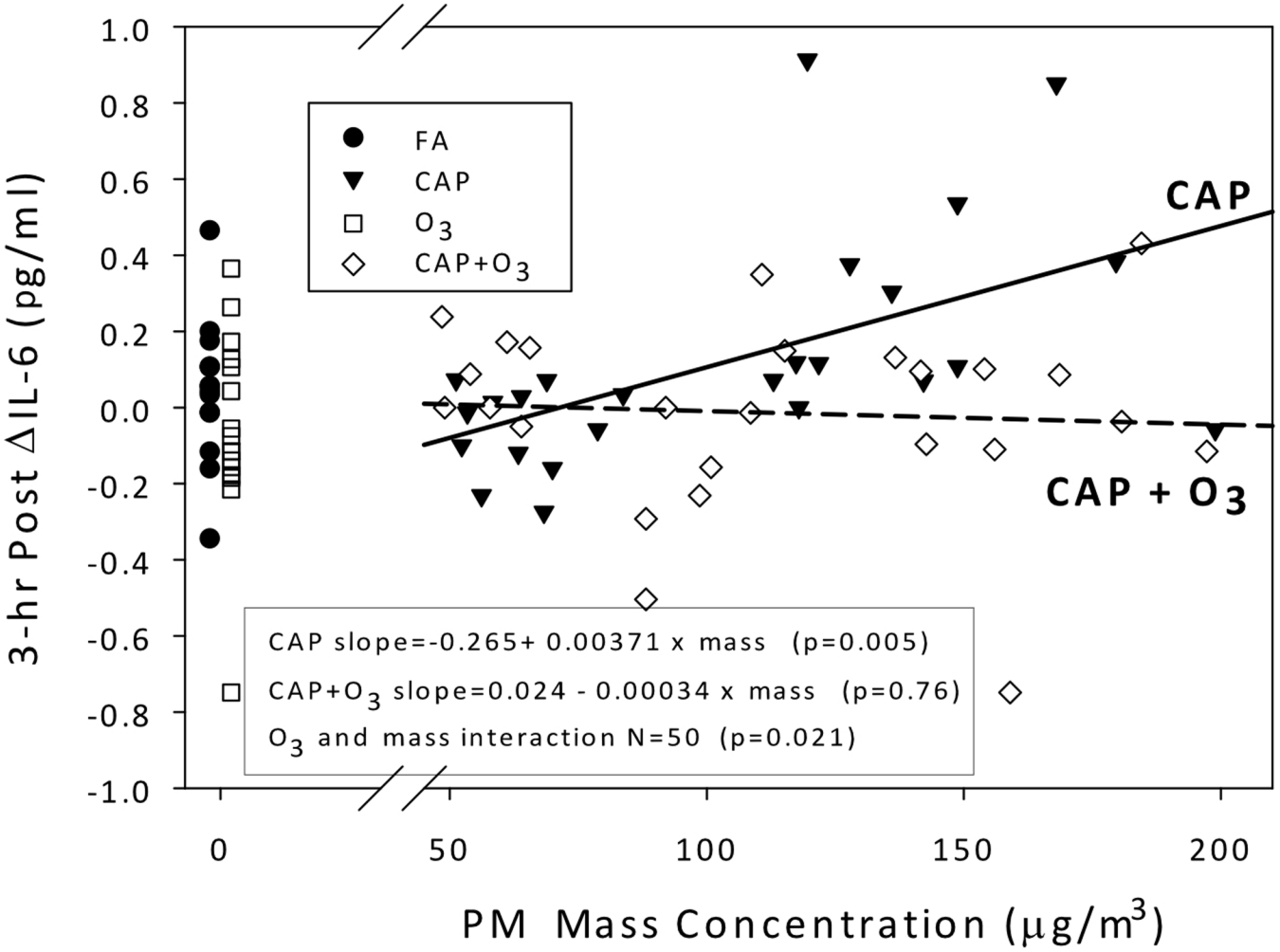

FIG 1.

Association between CAP mass concentration and blood IL-6 change 3-hrs post for CAP and CAP+O_3 exposures. Individual data are shown for 80 exposures. Data for FA (●) and O_3 (□) are shown separately to the left. A solid regression line is shown for CAP observations (▼) and dashed line for CAP+O_3 observations (◊). There was a significant positive association between PM mass concentration and IL-6 change 3-hrs after for CAP but not for CAP+O_3 exposures, with significant effect modification by ozone. The 3-hr post ΔIL-6= 3-hr IL-6 minus pre-exposure IL-6. Abbreviations: FA, filtered air; CAP, concentrated ambient particle; O_3, ozone; PM, particulate matter.

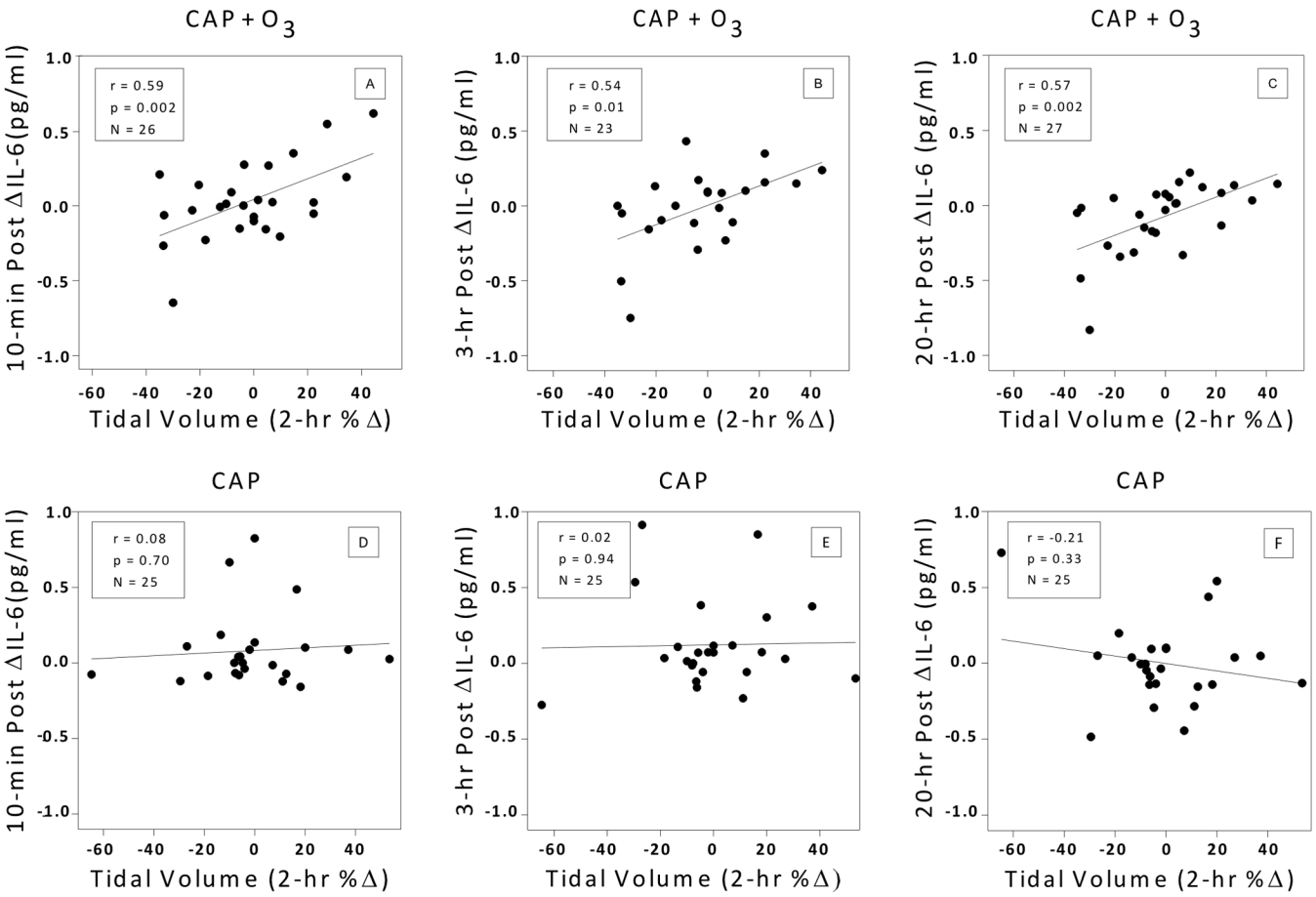

FIG 2.

Association between tidal volume 2-hr percent change and blood IL-6 change post exposure. Individual data are shown. Regression lines are shown by solid lines. Pearson partial correlations coefficients are reported, controlling for repeated measures of $CAP_{<100}$ and $CAP_{\geq 100}$. (A–C) CAP+O_3 exposures: 10-min post; 3-hrs post; and 20-hrs post. (D–F) CAP exposures: 10-min post; 3-hrs post; and 20-hr post. There were significant positive associations between tidal volume percent change during CAP+O_3 exposures and IL-6 changes at all three time points after but not for CAP alone exposures. Tidal volume 2-hr %Δ= 100 × [(2-hr minus 0-hr) / 0-hr]. Post ΔIL-6= post IL-6 minus pre-exposure IL-6. Abbreviations: CAP, concentrated ambient particle; O_3, ozone.

Table 1

Participant Exposure Order Groupings.

	No Ozone Group			Ozone Group		
Participants	**FA**	**CAP_{60}**	**CAP_{150}**	**$FA+O_3$**	**$CAP_{60}+O_3$**	**$CAP_{150}+O_3$**
4 N, 3 A	X	X	X			
4 N, 4 A[†]				X	X	X
2 N	X		X	X		X
3 N, 3 A	X	X	X	X	X	X
Exposure totals:	15	13	15	16	13	16

Abbreviations: FA, filtered air; CAP_{60}, concentrated ambient particle, target 60 μg/m^3; CAP_{150}, concentrated ambient particle, target 150 μg/m^3; O_3, ozone; N, non-asthmatic; A, asthmatic.

[†]One participant did not complete the $CAP_{60}+O_3$ exposure.

Table 2

Timeline of Outcome Measurements for the 3-day Exposure Protocol.

Time	Day 1	Day 2	Day 3
9 am	flow-volume	**blood** *(pre)*	**blood** *(20-hr post)*
	PC_{20}†	D_LCO/lung volumes	flow-volume
	sputum *(pre)*	flow-volume	PC_{20}†
			sputum *(20-hr post)*
11 am – 1 pm		**2-hr exposure**††	
1:10 pm		**blood** *(10-min post)*	
		flow-volume	
		D_LCO/lung volumes	
3 pm		30-min exercise test	
		flow-volume	
4 pm		sputum *(3-hr post)*	
		blood *(3-hr post)*	

Abbreviations: PC_{20}, methacholine challenge provocation concentration causing a 20% decrease in forced expiratory 1-sec volume from control; D_LCO, lung diffusion capacity for carbon monoxide.

The post exposure time in brackets is the time the test was carried out with reference to the end of the 2-hr exposure.

† Asthmatics only.

†† Respiratory minute ventilation and frequency measured at start and every 30 minutes thereafter.

Table 3

Participant Physical Characteristics, Baseline Spirometry and Mediators.

	Asthmatics	Non-Asthmatics
Gender (male/female)	4/6	7/6
Age (years)	27.5 ± 1.9	26.5 ± 1.5
BMI (kg/m^2)	21.6 ± 0.9	22.9 ± 0.7
Skin prick (# positive)	2.5	1.8
PC_{20} (mg/ml)	2.2	> 8
FEV_1 (%pred)	90.4 ± 2.5	99.3 ± 3.4
FVC (%pred)	96.7 ± 2.6	98.2 ± 2.9
FEV_1/FVC (%)†	78.9 ± 2.2	84.9 ± 1.5
Blood TNF-α (pg/ml)	0.10 ± 0.02	0.16 ± 0.04
IL-6 (pg/ml)	0.42 ± 0.06	0.66 ± 0.17

Values reported as means ± SEs.
Skin prick test is # allergens with wheal diameter ≥ 3 mm.

Abbreviations: BMI, body mass index; PC_{20}, see Table 2; FEV_1, forced expiratory 1-sec volume; FVC, forced vital capacity; %pred, percent of predicted normal values (according to Hankinson et al. 1999).

†Unpaired t-test, p=0.03, asthmatics versus non-asthmatics.

Table 4

Pollutant Levels and Environmental Conditions during Exposures.

	No Ozone Group			Ozone Group		
	FA	**$CAP_{<100}$**	**$CAP_{\geq 100}$**	**$FA+O_3$**	**$CAP_{<100}+O_3$**	**$CAP_{\geq 100}+O_3$**
Observations	15	12	16	16	12	17
Pollutants						
$PM_{2.5}$ (μg/m^3)	8±2	64±3	140±6	2±1	68±5	142±7
O_3 (ppb)	2±1	6±2	5±1	117±1	119±1	119±1
NO (ppb)	31±9	20±3	29±6	9±2	6±1	9±2
NO_2 (ppb)	21±2	18±1	22±2	52±9	34±3	50±6
Inorganic Ions						
NH_4^+ (μg/m^3)	0.0±0.1	2.4±2.0	6.2±4.7	0.0±0.1	6.0±9.3	6.4±4.1
SO_4^{2-} (μg/m^3)	0.0±0.1	7.2±5.6	17.6±12.0	0.1±0.2	11.5±9.4	19.3±12.4
NO_3^- (μg/m^3)	0.0±0.2	2.4±1.6	14.2±11.7	0.1±0.2	7.8±14.6	14.9±11.2
Environmental						
Temp (°C)	23.6±0.3	24.4±0.3	24.1±0.3	23.7±0.2	23.5±0.3	23.6±0.3
RH (%)	22±2	28±4	26±3	25±3	36±4	24±4

Abbreviations: FA, filtered air; $CAP_{<100}$ and $CAP_{\geq 100}$, concentrated ambient particle exposures with a gravimetric 2-hr integrated mass concentration <100 and ≥100 μg/m^3; $PM_{2.5}$, particulate matter with an aerodynamic diameter <2.5 μm; O_3, ozone; NO, nitric oxide; NO_2, nitrogen dioxide; NH_4^+; ammonium; SO_4^{2-}, sulphate; NO_3^-, nitrate; temp/RH, temperature and relative humidity.

Note: Filter sample ($PM_{2.5}$/inorganic ions), gaseous pollutants and temp/RH were taken from the FA/CAP airstream delivered to the subject.
For all exposures, SO_2 and CO levels were below 5.1 ppb & 0.6 ppm, respectively.

Table 5

Tidal Volume 2-hr Percent Change by 3-hr Post Exposure Blood IL-6 Changes, for Ozone and no Ozone Exposures.

	Ozone Exposures†		No Ozone Exposures††, ##	
Tidal Volume	**IL-6 decrease**	**IL-6 decrease ≥0**	**IL-6 increase**	**IL-6 decrease ≥0**
V_T decrease	14 (70.0%)	6 (30.0%)	9 (40.9%)	13 (59.1%)
V_T increase ≥0	6 (31.6%)	13 (68.4%)	4 (23.5%)	13 (76.5%)

Abbreviations: V_T, tidal volume.

Values are number of exposures (percent of row total).
Tidal volume 2-hr percent change= 100 × [(2-hr V_T minus 0-hr V_T)/0-hr V_T].
IL-6 3-hr post exposure change= 3-hr IL-6 minus pre-exposure IL-6.

† Fisher's Exact test: Odds Ratio= 5.1, p=0.026 (n=39).

†† Fisher's Exact test: Odds Ratio= 2.3, p=0.32 (n=39).

Breslow-Day comparison of two odds ratios p=0.42.