Personal and Ambient Air Pollution is Associated with Increased Exhaled Nitric Oxide in Children with Asthma

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BACKGROUND: Research has shown associations between pediatric asthma outcomes and airborne particulate matter (PM). The importance of particle components remains to be determined.

METHODS: We followed a panel of 45 schoolchildren with persistent asthma living in Southern California. Subjects were monitored over 10 days with offline fractional exhaled nitric oxide (FENO), a biomarker of airway inflammation. Personal active sampler exposures included continuous particulate matter < 2.5 µm in aerodynamic diameter (PM₂.₅), 24-hr PM₂.₅ elemental and organic carbon (EC, OC), and 24-hr nitrogen dioxide. Ambient exposures included PM₂.₅, PM₁₀ EC and OC, and NO₂. Data were analyzed with mixed models controlling for personal temperature, humidity and 10-day period.

RESULTS: The strongest positive associations were between FENO and 2-day average pollutant concentrations. Per interquartile range pollutant increase, these were: for 24 µg/m³ personal PM₂.₅, 1.1 ppb FENO (95% confidence interval (CI), 0.1–1.9); for 0.6 µg/m³ personal EC, 0.7 ppb FENO (95% CI, 0.3–1.1); for 17 ppb personal NO₂, 1.6 ppb FENO (95% CI, 0.4–2.8). Larger associations were found for ambient EC and smaller associations for ambient NO₂. Ambient PM₂.₅ and personal and ambient OC were significant only in subjects taking inhaled corticosteroids (ICS) alone. Subjects taking both ICS and antileukotrienes showed no significant associations. Distributed lag models showed personal PM₂.₅ in the preceding 5 hr was associated with FENO. In two-pollutant models, the most robust associations were for personal and ambient EC and NO₂, and for personal but not ambient PM₂.₅.

CONCLUSION: PM associations with airway inflammation in asthmatics may be missed using ambient particle mass, which may not sufficiently represent causal pollutant components from fossil fuel combustion.

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co-regressed with ambient EC or OC fractions of PM$_{10}$ but larger positive associations of EC and OC with symptoms remained stable. This finding suggests that combustion-related organic compounds such as those in diesel exhaust are causally related to acute asthma. EC and OC may have represented particle components (e.g., PAHs) and key sources that EC and OC may have represented particle components (e.g., PAHs) and key sources that co-regressed with ambient EC or OC fractions of PM$_{10}$ but larger positive associations of EC and OC with symptoms remained stable. This finding suggests that combustion-related organic compounds such as those in diesel exhaust are causally related to acute asthma. EC and OC may have represented particle components (e.g., PAHs) and key sources that.

**Materials and Methods**

**Design and population.** This panel study involves repeated daily measurements of health outcomes and exposures in children with asthma. It was conducted in urban regions of Southern California with high levels of air pollution, primarily from mobile sources of fossil fuel combustion. The first region was in Riverside, California, where subjects were followed from August through mid-December 2003. This is a downwind smog receptor site just inland from LA County. The second region was in Whittier, California, in eastern LA County, where subjects were followed from July through November 2004. Data reported in this article are from an intensive phase of study over 10-day periods. This involved having subjects wear an active personal monitor to measure exposure to ambient pollutants, and collection of an off-line measurement of FENO at the end of each 24 hr of personal sampling. Data for both FENO and personal air pollutant exposures were collected from 48 asthmatic children (16 from Riverside) over twelve 10-day periods, four subjects per period. Subjects were followed daily in their homes to check the validity of exposure run. Empirical variograms (graphical measures of the correlation between observations as a function of time) showed an autoregressive-1 correlation structure adequately described the observed variability. All exposures were mean-centered by individual to obtain comparability from one participant location to another.

**Exposures.** Our personal exposure monitor was constructed to collect 24-hr samples of personal PM$_{2.5}$, EC and OC fractions of PM$_{2.5}$, NO$_2$, temperature, and relative humidity (RH). Each subject wore the monitor during waking hours in a backpack. Personal PM$_{2.5}$ monitors operated at 4 L/min with the sampling inlet near the breathing zone. One-minute average PM$_{2.5}$ was measured using an integrated nephelometer, the personal DataRAM model 1200 (pDR; MIE Inc., Bedford, MA). Compliance (motion), RH, and temperature loggers recorded at 1-min intervals (Onset Computer Corp., Pocasset, MA). Data were downloaded and checked for quality and compliance daily. pDR data were adjusted for personal RH (Chakrabarti et al. 2004). The pDR configuration also included a filter cartridge for EC and OC. A 2.5-μm sharp-cut cyclone was attached upstream of the pDR, and PM$_{2.5}$ was collected on 37-mm quartz filters (Whatman Inc., Florham Park, NJ). The personal PM$_{2.5}$ monitor was validated by our group in a previous report and it is described in detail elsewhere (Chakrabarti et al. 2004).

We developed an active sampling system for NO$_2$ that ran in parallel with the pDR. It uses a miniaturized diaphragm pump (Virtual Industry VMP1625, Colorado Springs, CO) run at 0.1 L/min, and triethanolamine-treated molecular sieve sorbent tubes as a collection medium (SKC West Inc., Fullerton, CA). NO$_2$ measurements were based on National Institute for Occupational Safety and Health method 6014 (National Institute for Occupational Safety and Health 1994). This personal NO$_2$ exposure monitor was validated by our group in a previous report and it is described in detail elsewhere (Staiger et al. 2005).

Central-site ambient exposures included 24-hr PM$_{2.5}$, PM$_{10}$, and NO$_2$ EC and OC collected from early evening to early evening with Harvard Impactors (Air Diagnostics and Engineering, Inc., Naples, ME) using standard procedures. After sample collection on quartz filters, particulate carbon was speciated into OC and EC using the thermal manganese dioxide oxidation technique (for details, see Fung et al. 2002). EC and OC are expressed as amount of total carbon per sample. Central-site data also included hourly ozone, NO$_2$, and CO measured by the South Coast Air Quality Management District. In Riverside, the district center was centrally located. In Whittier, we constructed a central site at a subject home elevated on a hill. Data for O$_3$, NO$_2$, and CO came from two district sites at opposite ends of the Whittier study region (La Habra and Pico Rivera). Hourly concentrations for the two stations were averaged.

**Statistical methods.** We used linear mixed-effects models (Verbeke and Molenberghs 2001) to estimate the association between each air pollutant and FENO. Because the data represent repeated measures on individuals over time, correlation among outcomes was present. We assumed a two-stage hierarchical model with random effects at the subject level, nested within exposure run. Empirical variograms (graphical measures of the correlation between observations as a function of time) showed an autoregressive-1 correlation structure adequately described the observed variability. All exposures were mean-centered by individual to obtain comparability from one participant location to another.
another (Sheppard et al. 2005). We investigated exposures preceding the \( F_{\text{ENO}} \) measurement including the last 24 hr (lag 0), the average of the 25th through 48th hour preceding the \( F_{\text{ENO}} \) measurement (lag 1), and a cumulative 2-day moving average (2-day MA). Results are expressed as ppb change in \( F_{\text{ENO}} \) per inter-quartile range (IQR) increase in pollutant.

Given their potential for confounding associations, we decided a priori that personal temperature, personal RH, and 10-day exposure run should be adjusted for in all models. We also tested potential confounding by respiratory infections, region of study, sex, cumulative daily use of as-needed \( \beta \)-agonist inhalers, and weekend. None of these variables confounded associations, and there was no air pollutant interaction with sex or respiratory infection (only 13 person-days reported).

Secondary analyses examining potential effect modification by medication use were also conducted. Residual diagnostics were conducted for all models to investigate the presence of influential data points and deviations from assumed functional form. No data points were removed for influence. As a validity check to the likelihood assumptions made by the linear mixed-effects model, regression models using generalized estimating equations (Diggle et al. 2002) in combination with robust standard error estimates were also fit. No qualitative differences in our study results were found.

To investigate the lag effect of hourly personal PM2.5 on \( F_{\text{ENO}} \), we used a fourth-order polynomial distributed lag mixed-effects model (Schwartz 2000). An autoregressive correlation structure was assumed and supported by residual diagnostics. Distributed lag models stratifying on anti-inflammatory medication use were also fit.

Between-pollutant confounding was tested with two-pollutant regression models. We compared the change in regression parameters from single- to two-pollutant models for the same subset of nonmissing person-days for both pollutants. Interaction between pollutants was tested first in a regression equation with the two pollutant variables and their product term as predictors.

### Results

**Descriptive subject data.** The Riverside panel included eight 10-day runs with four subjects per run. However, the Sievers NO Analyzer malfunctioned after the third day of run 5. The analyzer was replaced by a new one, but not until after the end of Riverside run 8. In addition, personal samplers malfunctioned during most of the entire first run for three subjects. This was resolved before the next run. Therefore, we retained for analysis data from the first four 10-day runs in Riverside involving 13 subjects. Data were collected for all

### Table 1. Study group characteristics, Riverside and Whittier, California, asthma panels.

| Subject variable | Data |
|------------------|------|
| Age [years, mean (range)] | 13.5 (9–18) |
| Sex [no. (%)] | Female 14 (31) Male 31 (69) |
| Race [no. (%)] | Hispanic 26 (58) White 14 (31) Black 5 (11) |
| No. (%) with mean percent FEV1 < 80% | 11 (24) |
| Race [no. (%)] | Black 5 (11) White 14 (31) Hispanic 26 (58) |
| Sex [no. (%)] | Female 14 (31) Male 31 (69) |
| Age [years, mean (range)] | 13.5 (9–18) |

### Table 2. Distribution of \( F_{\text{ENO}} \) (ppb) by region and medication use.

| Variable | Mean ± SD | Median 25–75th percentile | Range |
|----------|-----------|--------------------------|-------|
| Overall (45 subjects) | 25.6 ± 25.1 | 18.2 | 10.5–32.0 | 2.7–154 |
| Region | | | | |
| Riverside (13 subjects) | 16.6 ± 11.1 | 13.6 | 8.9–18.8 | 3.4–48.5 |
| Whittier (32 subjects) | 29.4 ± 28.1 | 20.6 | 10.5–33.3 | 2.7–154 |
| Medication group | | | | |
| No anti-inflammatory medication (14 subjects) | 35.9 ± 35.8 | 23.9 | 11.8–46.6 | 5.0–154 |
| Anti-inflammatory medication (31 subjects) | 21.1 ± 16.8 | 17.5 | 9.9–26.2 | 2.7–98.4 |
| Anti-inflammatory medication group | | | | |
| Inhaled corticosteroids (13 subjects) | 20.4 ± 19.5 | 16.0 | 6.7–22.1 | 2.7–98.4 |
| Antileukotrienes ± inhaled corticosteroids (12 subjects) | 22.4 ± 19.7 | 19.6 | 14.5–30.7 | 5.0–51.4 |

### Table 3. Daily air measurements stratified by study panel.

| Exposure | Riverside panel (n = 13) | Whittier panel (n = 32) |
|----------|--------------------------|------------------------|
| Personal exposure | | |
| 24-hr PM\(_2.5\) (µg/m\(^3\)) | 96 (11) | 32.78 ± 21.84 | 28.14 | 28.41 | 7.27/98.43 |
| 1-hr max PM\(_2.5\) (µg/m\(^3\)) | 96 (14) | 97.94 ± 70.29 | 83.7 | 86.75 | 7.55/98.43 |
| 8-hr max PM\(_2.5\) (µg/m\(^3\)) | 96 (14) | 47.21 ± 30.9 | 38.5 | 45.4 | 8.9/132.1 |
| 24-hr PM\(_2.5\) EC (µg/m\(^3\)) | 100 (9) | 0.42 ± 0.69 | 0.34 | 0.32 | 0.01/6.94 |
| 24-hr PM\(_2.5\) OC (µg/m\(^3\)) | 100 (9) | 5.63 ± 2.59 | 4.98 | 3.36 | 1.94/31.68 |
| 24-hr NO\(_2\) (ppb) | 107 (2) | 24.26 ± 9.34 | 24.31 | 11.59 | 5.16/47.61 |
| 24-hr temperature | 106 (3) | 79.88 ± 3.75 | 79.06 | 6.14 | 71.33/83.85 |
| Central site (PM; µg/m\(^3\)) | | | | | |
| 24-hr PM\(_10\) | 96 (10) | 36.63 ± 23.46 | 29.26 | 30.83 | 5.92/67.22 |
| 24-hr PM\(_10\) | 100 (9) | 70.82 ± 29.36 | 65.96 | 40.38 | 30.75/154.05 |
| 24-hr PM\(_2.5\) EC | 94 (15) | 1.61 ± 0.78 | 1.35 | 0.91 | 0.52/3.64 |
| 24-hr PM\(_2.5\) OC | 94 (15) | 6.88 ± 1.86 | 6.07 | 2.19 | 1.41/11.62 |
| Central site (gases; ppb) | | | | | |
| 8-hr max O\(_3\) | 100 (9) | 76.37 ± 18.47 | 73.62 | 18.62 | 33.38/120.75 |
| 8-hr max NO\(_2\) | 100 (9) | 27.39 ± 14.6 | 28.41 | 16.14 | 17.75/42.73 |
| 8-hr max CO | 100 (9) | 530.6 ± 369.58 | 442.86 | 514.29 | 0.1/1324.86 |

**Abbreviations:** max, maximum; min, minimum.
eight 10-day runs in Whittier for 32 subjects and combined with data from Riverside. Table 1 shows characteristics of the 45 subjects.

Of 446 daily pairs of FENO samples, we found that 372 pairs (83%) were reliable by our criteria (± 3 ppb NO or ± 10% difference). In the Supplemental Material (http://www.epplnline.org/docs/2006/9141/suppl.pdf), we present analyses of relationships of FENO with air pollutants. In addition, there was no relationship between indoor NO and acceptable FENO pairs (slope 0.04 ± 0.03, p = 0.21), and indoor NO concentration did not influence associations of air pollutant exposures with FENO.

The distribution of FENO concentrations by region and medication use is shown in Table 2. Subjects in Whittier had nonsignificantly higher FENO than did Riverside subjects. As confirmed in daily diaries and by research staff, 14 subjects were not taking anti-inflammatory controller medications and 31 were [inhaled corticosteroids (ICS) and antileukotrienes (leukotriene receptor antagonists zafirlukast and montelukast)]. Subjects not taking any anti-inflammatory medication had higher mean FENO, consistent with expectations (Zeidler et al. 2004). There was no difference within the anti-inflammatory medication group by use of antileukotrienes.

Descriptive exposure data. Table 3 shows descriptive data for exposures by region. Central-site particle mass, EC, OC, NO2, and O3 concentrations were higher in Riverside because this warmer receptor region is located downwind of major urban sources in LA, adding to local sources of these air pollutants. Despite lower ambient concentrations, personal EC, OC, and NO2 were somewhat higher in Whittier than in Riverside, perhaps reflecting proximity of subjects to densely populated LA areas having a higher local traffic impact. Maximum 1-hr personal PM2.5 fanged up to 573 µg/m3.

Table 4 shows the between-pollutant correlations. Correlations of personal PM2.5 with personal EC, OC, and NO2 were significant but small, and not much different from correlations with ambient data. Personal EC and OC were not correlated with ambient EC and OC but were correlated with ambient NO2. Personal PM2.5 was moderately correlated with ambient PM2.5 (r = 0.64), and personal NO2 was moderately correlated with ambient NO2 (r = 0.46). Ambient exposures were all moderately correlated with each other.

Regression models for personal as compared with central-site air pollutants. We found positive associations of FENO with increasing personal PM2.5 mass, EC, and NO2, and NO2 were stronger and more significant in the group on anti-inflammatory medications than in the group not on anti-inflammatory medications. However, product terms for these pollutants by a medication group indicator were not significant at p < 0.1. The anti-inflammatory medication group was next split into subjects only taking ICS and subjects taking antileukotrienes with or without ICS. Results suggest effect modification by antileukotrienes combined with ICS. Among the taking ICS but not antileukotrienes, all air pollutants were significantly associated with FENO, or nearly so in the case of ambient PM10. Personal and ambient OC and ambient PM2.5 were significantly associated with FENO only in the group taking ICS but not antileukotrienes. Product terms for controller medication group by personal PM2.5 and by ambient PM2.5 and PM10 were all significant. We found no meaningful change in the null results dropping two of the 12 subjects taking antileukotrienes who were not also taking ICS.

Distributed lag models for hourly personal PM2.5. Figure 1 shows that FENO in all 45 subjects is acutely associated with PM2.5 exposure in the 5 hr preceding measurement, with the lower 95% confidence bound crossing

|  | Personal | Central |
|---|---|---|
|  | PM2.5 | EC | OC | NO2 | PM2.5 | EC | OC | NO2 |
| 24-hr personal PM2.5 | 1.00 | 0.18** | 0.15* | 0.33** | 0.64** | 0.12* | 0.21** | 0.22** |
| 24-hr personal EC | 1.00 | 0.41** | 0.21** | 0.00 | 0.04 | 0.01 | 0.23** |
| 24-hr personal NO2 | 1.00 | 0.20** | 0.11* | 0.03 | 0.02 | 0.21** |
| 24-hr central PM2.5 | 1.00 | 0.55** | 0.66** | 0.12* | 0.19** | 0.17** | 0.48** |
| 24-hr central EC | 1.00 | 0.95** | 0.95** | 0.87** | 0.70** |
| 24-hr central NO2 | 1.00 | 0.62** |

*p-value < 0.05; **p-value < 0.001 from Wald-based tests of Spearman correlation coefficients.

Table 5. Mixed-model estimates of the association between personal and central-site air pollutant exposure and FENO.

| Exposure | Personal | Central site |
|---|---|---|
| | Coefficient (95% CI) | p-Value | Coefficient (95% CI) | p-Value |
| PM2.5 | | | | |
| Lag 0 | 0.42 (–0.15 to 0.99) | 0.148 | 0.03 (–0.68 to 0.74) | 0.925 |
| Lag 1 | 0.51 (–0.10 to 1.12) | 0.100 | 0.44 (–0.28 to 1.16) | 0.226 |
| 2-day MA | 1.01 (0.14 to 1.88) | 0.024 | 0.52 (–0.43 to 1.47) | 0.287 |
| PM2.5 EC | | | | |
| Lag 0 | 0.29 (0.10 to 0.48) | 0.003 | 0.10 (–0.65 to 0.85) | 0.793 |
| Lag 1 | –0.01 (–0.23 to 0.21) | 0.898 | 0.99 (0.27 to 1.71) | 0.007 |
| 2-day MA | 0.72 (0.32 to 1.12) | 0.001 | 1.38 (0.15 to 2.61) | 0.027 |
| PM2.5 OC | | | | |
| Lag 0 | 0.51 (–0.29 to 1.30) | 0.207 | 0.93 (–0.20 to 2.06) | 0.104 |
| Lag 1 | 0.13 (–0.77 to 1.03) | 0.768 | 0.51 (–0.64 to 1.68) | 0.398 |
| 2-day MA | 0.94 (–0.47 to 2.35) | 0.190 | 1.61 (–0.17 to 3.37) | 0.077 |
| NO2 | | | | |
| Lag 0 | 0.25 (–0.44 to 0.94) | 0.471 | 0.10 (–0.55 to 0.75) | 0.752 |
| Lag 1 | 0.60 (–0.12 to 1.32) | 0.103 | 0.72 (0.06 to 1.36) | 0.028 |
| 2-day MA | 1.83 (0.43 to 2.25) | 0.008 | 1.36 (0.39 to 2.33) | 0.006 |

CI, confidence interval. Lag 0: 24-hr average preceding the FENO measurement; Lag 1: average for the 25th through 48th preceding the FENO measurement; 2-day MA: moving average for the 48 hr preceding the FENO measurement.

*The expected change in FENO associated with one IQR change in each air pollutant level, adjusted for personal temperature, personal relative humidity, and run. IQRs for personal air pollutant measurements were 24 µg/m3 for PM2.5, 0.6 µg/m3 for PM2.5 EC, 4.1 µg/m3 for PM2.5 OC, and 17 ppb for NO2. IQRs for central-site air pollutant measurements were 15 µg/m3 for PM2.5, 0.6 µg/m3 for PM2.5 EC, 2.8 µg/m3 for PM2.5 OC, and 12 ppb for NO2.
null after lag 5 and coefficients remaining near zero after that. Because 24 hr, we found no significant association between PM2.5 and FENO partly because of the high variability resulting from such large lag times. The associations are stronger the closer the personal PM2.5 measurement is to the FENO measurement. At lag 0 hr, FENO was estimated to increase 0.46 ppb (95% CI, 0.13–0.78) per one-IQR increase in personal PM2.5 (24 µg/m3).

Table 2 shows that associations of FENO with lag 0–5 hr personal PM2.5 were moderately stronger in the group not on anti-inflammatory medications. At lag 0 hr, FENO increased 0.57 ppb (95% CI, –0.06 to 1.20) per IQR increase in personal PM2.5 (24 µg/m3) among subjects not taking anti-inflammatory medications (Figure 2A), compared to 0.35 ppb (95% CI, –0.02 to 0.71) among subjects taking anti-inflammatory medications (Figure 2B). When the medication group was further divided based on use of antileukotrienes, we found an early association between PM2.5 and FENO (0.54 ppb; 95% CI, 0.05–0.87), but the difference was not significant (p = 0.14).

Two-pollutant models. The only significant interaction between air pollutants tested in the two-pollutant models was a positive interaction between personal PM2.5 and personal OC (p = 0.003). Figure 3 shows models for personal exposures and separately for ambient exposures. For personal two-pollutant models, the most robust association was for EC, followed by NO2, PM2.5, and then OC. For two-pollutant models using ambient data, we found CIs widened for both pollutants, partly due to their correlation (Table 3). Co-regression of ambient PM2.5 with ambient EC, OC, or NO2 led to a large reduction in the parameter for PM2.5 to near zero, but little change in estimates of association for EC, OC, or NO2. Ambient OC was marked confounded by both NO2 and EC. Overall, the ambient two-pollutant models show the most robust association for NO2 and EC.

To assess whether the association of FENO in all 45 subjects with ambient EC or NO2 was independent of personal EC or NO2, we co-regressed two-day average ambient with personal EC (not correlated; Table 4), and personal with ambient NO2 (moderately correlated). Associations for personal and central-site EC were completely stable compared with single-pollutant models (Table 5) [0.73 ppb (95% CI, 0.30–1.15) and 1.37 ppb (95% CI, 0.13–2.60), respectively]. Personal NO2 completely confounded ambient NO2 [1.46 ppb (95% CI, –0.24 to 3.16) and 0.19 ppb (95% CI, 1.24 to 1.62), respectively].

To assess whether the positive association of FENO in 19 subjects on ICS with ambient PM2.5 or OC was independent of personal PM2.5 or OC, we co-regressed ambient with personal exposures for the same pollutant. The association with ambient PM2.5 was completely confounded by personal PM2.5, which showed nearly the same positive association with FENO as the single-pollutant model in Table 6. The between-pollutant correlation was 0.53. Similarly, the association with ambient OC was halved, whereas personal OC was minimally reduced by 13%. The between-pollutant correlation was nonsignificant. The isolated association of ambient PM2.5 with FENO in this group was independent of ambient EC, OC, and NO2.

Discussion

Positive associations were found for FENO in relation to personal and ambient air pollutants, with evidence from the multiple-pollutant approach that traffic-related sources of air pollutants underlie the findings. Although the estimates of effect were small (≤ 2.5 ppb FENO), inasmuch as FENO is a marker of airway inflammation, this would suggest that air pollution increases inflammation. We cannot say whether the chosen estimate of effect by the interquartile range increase in air pollution is clinically relevant or not.

Other studies have found associations of ambient air pollutants with FENO in elderly adults with asthma (Jansen et al. 2005), adults with cardiac disease (Adamkiewicz et al. 2004), healthy adults (van Amsterdam et al. 1999), and general populations of schoolchildren (Fischer et al. 2002; Steerenberg et al. 2001, 2003). Our findings are most consistent with a panel study of 19 asthmatic children in Seattle, Washington (Koenig et al. 2003). In nine children not taking ICs, they found an approximately 4-ppb increase in FENO per 10-µg/m3 lag 0 personal, indoor and outdoor home, and ambient PM2.5. In a follow-up report of the Seattle panel study, Koenig et al. (2005) found that the estimated ambient-generated fraction of personal PM2.5 exposure was positively associated with FENO, but not the estimated indoor-generated fraction.

Results of models using distributed hourly lags and daily average personal PM2.5 point to

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Table 6. Mixed-model estimates of associations between 2-day moving average personal and central-site air pollutant exposures and FENO stratified by medication use.

| Exposure | Not taking anti-inflammatory medications (14 subjects) | Taking anti-inflammatory medications (31 subjects) | Inhaled corticosteroids (19 subjects)a | Antileukotrienes ± inhaled corticosteroids (12 subjects)b |
|----------|------------------------------------------------------|--------------------------------------------------|---------------------------------------|----------------------------------------------------------|
| Personal PM2.5 | 1.11 (1.39 to 3.60) | 0.380 | 0.19 (1.84) | 0.17 | 1.58 (0.72 to 2.43) | 0.0004 | 0.89 (–2.73 to 0.95)* | 0.339 |
| PM2.5 EC | 1.04 (0.28 to 1.01) | 0.031 | 0.71 (1.28 to 1.50) | 0.001 | 0.67 (0.28 to 1.07) | 0.003 | 0.52 (–1.98 to 3.02) | 0.682 |
| PM2.5 OC | 0.88 (1.52 to 3.39) | 0.484 | 0.87 (0.79 to 2.53) | 0.302 | 2.47 (0.30 to 4.64) | 0.026 | 1.73 (–0.70 to 4.16) | 0.160 |
| NO2 | 0.80 (–3.01 to 4.61) | 0.677 | 1.67 (0.55 to 2.79) | 0.004 | 1.22 (0.04 to 2.40) | 0.043 | 1.73 (–0.70 to 4.16) | 0.160 |
| Central site PM2.5 | 0.44 (–0.15 to 2.53) | 0.677 | 0.55 (–0.47 to 1.57) | 0.290 | 1.16 (0.11 to 2.20) | 0.030 | 0.75 (–2.83 to 1.32)* | 0.471 |
| PM2.5 EC | 0.76 (1.54 to 3.07) | 0.51 | 0.53 (0.83 to 1.90) | 0.439 | 1.28 (–0.01 to 2.58) | 0.053 | –2.10 (–5.33 to 1.12)* | 0.196 |
| PM2.5 OC | 1.02 (–2.50 to 4.60) | 0.70 | 1.42 (0.25 to 2.60) | 0.018 | 1.28 (0.07 to 2.49) | 0.038 | 1.15 (–1.58 to 3.88) | 0.403 |
| NO2 | 0.36 (–0.47 to 2.79) | 0.207 | 2.05 (0.24 to 3.86) | 0.026 | 1.96 (0.14 to 3.78) | 0.035 | 1.29 (–2.58 to 5.15) | 0.308 |

*a Two subjects were also taking inhaled cromolyn. b Two subjects were taking antileukotrienes only, and 10 were taking antileukotrienes plus inhaled corticosteroids. The expected change in FENO associated with a 1-IQR change in each 2-day moving average air pollutant, adjusted for personal temperature, personal relative humidity, and run. IQR for personal air pollutant measurements were 24 µg/m3 for PM2.5, 0.6 µg/m3 for PM2.5 EC, 4.1 µg/m3 for PM2.5 OC, and 17 ppb for NO2. IQR for central-site air pollutant measurements were 15 µg/m3 for PM2.5, 23 µg/m3 for PM2.5 EC, 2.9 µg/m3 for PM2.5 OC, and 12 ppb for NO2. p < 0.05 for difference with the coefficient estimate for subjects taking inhaled corticosteroids but not antileukotriene medication.
possible cumulative and lag effects (48-hr mean and 1-day lag) and more immediate effects (previous 5 hr) on FENO. Findings in other panel studies also support a rapid effect of recent hourly PM$_{2.5}$ on biomarkers of inflammation in children with asthma, including the aforementioned Seattle study using FENO (Mar et al. 2005), and a Denver, Colorado, study using urinary leukotriene E$_4$ (Rabinovitch et al. 2006). This may reflect acute-phase inflammation from an early release of mediators by mast and other cells, followed by a late-phase response peaking a few hours later and characterized by lymphocyte activation and infiltration (Hamid et al. 2003). However, experimental data are needed to provide evidence that is more definitive for a delayed (or cumulative) effect from multiday exposures.

The generally more robust associations for personal than for central-site PM$_{2.5}$ exposures suggest that nondifferential exposure misclassification leads to attenuation of the particle association and increased variance. This is graphically demonstrated in Figure 3. A coherent contrast between personal and ambient PM$_{2.5}$ using FEV$_1$ as an outcome was found in our previous asthma panel study (Delfino et al. 2004). In addition, the present associations of FENO with outdoor ambient EC and OC but not ambient PM$_{2.5}$ suggest that PM associations may be missed using total particle mass measurements alone, coherent with our previous findings for ambient EC, OC, and symptoms in asthmatic children in Los Angeles (Delfino et al. 2003). Furthermore, the present significant associations of FENO with both personal and ambient EC and NO$_2$ suggest that despite potential misclassification of personal exposure, ambient data linked to combustion sources represents casual pollutant components.

**Figure 1.** Estimated lag effect of hourly personal PM$_{2.5}$ on FENO. Estimates are based on a 4th-degree linear mixed-effects polynomial distributed lag model with AR(1) correlation structure. Expected changes in FENO correspond to a 1-IQR (24 µg/m$^3$) change in PM$_{2.5}$. Blue bands indicate pointwise 95% CIs. Vertical dashes represent hourly measurements. All estimates are adjusted for personal temperature and relative humidity.

**Figure 2.** Estimated lag effect of hourly personal PM$_{2.5}$ on FENO by use of medications. (A) No anti-inflammatory medications. (B) Anti-inflammatory medications. (C) Inhaled corticosteroids. (D) Antileukotrienes and inhaled corticosteroids. Estimates are based on a 4th-degree linear mixed-effects polynomial distributed lag model with AR(11) correlation structure. Expected changes in FENO correspond to a 1-IQR (24 µg/m$^3$) change in PM$_{2.5}$. Blue bands indicate pointwise 95% CIs. Vertical dashes represent hourly measurements. All estimates are adjusted for personal temperature and relative humidity.

**Figure 3.** One- and two-pollutant models for change in FENO using 2-day MA personal (A) and central-site (B) pollutant measurements. Squares: single-pollutant model; triangles: two-pollutants models. Expected change in FENO is per IQR in the pollutant of interest with 95% CIs. All estimates are adjusted for personal temperature and relative humidity.
Both personal and ambient two-pollutant models showed that EC and NO$_2$ had the most robust associations against inclusion of other pollutants. However, in contrast to ambient data, personal exposure models showed little to no confounding of personal PM$_{2.5}$ by EC or NO$_2$, suggesting that other causal factors in personal PM exposures are not represented by EC or NO$_2$. Both EC and NO$_2$ originate in the study region largely from traffic-related sources, which have high spatial variability (Sioutas et al. 2005), thereby creating highly variable personal exposures to EC and NO$_2$ in the present study population. Indoor concentrations represent the collective contributions of local traffic and other outdoor sources, as well as indoor sources and activities, such as cooking. Similarly, outdoor OC is dominated by vehicular emissions and secondary organics, whereas indoor OC includes contributions from cooking and other sources of nontoxic semivolatile OC, in addition to outdoor OC that penetrates indoors. This, plus imprecision of the personal monitor for OC, may have increased misclassification of personal OC exposure and weakened associations. Personal PM$_{2.5}$, on the other hand, likely represents a variety of indoor particle components that can induce asthma, including allergens and endotoxin (Rabinovitch et al. 2005). It also represents ambient PM$_{2.5}$ given the moderate correlation between ambient and personal PM$_{2.5}$ ($R = 0.63$, $p < 0.001$). Interaction between personal PM$_{2.5}$ and OC suggests a possible interactive particle effect.

To assess further the potential contribution of ambient air pollution to associations of FEN$_{o}$ with personal exposures, we tested two-pollutant models with the same ambient and personal pollutant. Personal and ambient EC were independently associated with FEN$_{o}$, whereas personal NO$_2$ confounded the association of ambient NO$_2$ with FEN$_{o}$. Lack of correlation between personal and ambient EC suggests that different sources contribute to the concentrations. The moderate personal and ambient NO$_2$ correlation means that the same key sources contribute to both their concentrations and perhaps to effects, which were best captured by personal exposure measurements. Among the ICS-only group, personal PM$_{2.5}$ completely confounded ambient PM$_{2.5}$ and personal OC was similarly robust against ambient OC. Again, key sources were likely best captured by personal exposures measurements.

In Supplemental Material (http://www. ehponline.org/docs/2006/9141/suppl.pdf), we present possible reasons for the personal versus ambient pollutant findings in a preliminary assessment of the relationship of personal to indoor and outdoor home exposures.

There was no significant difference in the above associations between subjects taking anti-inflammatory medications versus those who were not. However, FEN$_{o}$ was associated with 2-day average personal and ambient NO$_2$, EC, OC, and PM$_{2.5}$, and ambient PM$_{10}$, among subjects taking ICS, but not in those taking a combination of ICS and antileukotrienes. Consistent differences were found for the last 5 hr of personal PM$_{2.5}$ exposure. This suggests a mixed pattern of susceptibility, because of either treatment regimen or greater ongoing asthma severity. Antileukotrienes are antagonists of type 1 cysteinyl leukotriene receptors. They are believed to have both anti-inflammatory as well as bronchodilator effects on the airways (Lipworth 1999). In a large clinical trial, improvements in most clinical asthma control measures occurred with either fluticasone or montelukast; but outcomes, especially FEN$_{o}$, improved significantly more with fluticasone than with montelukast treatment (Zeiger et al. 2006). The greater response to air pollutants despite medication suggests that the subjects taking ICS may be more susceptible perhaps as reflected by their persistent asthma. Persistent asthma is a diagnostic indication of the need for anti-inflammatory medication. The addition of antileukotrienes may have achieved additional control sufficient to blunt responses to air pollution exposures. Relevant experimental findings come from two clinical intervention studies showing reduced respiratory responses to air pollutants with montelukast treatment (Gong et al. 2001; Rundell et al. 2005). In addition, antileukotrienes are taken orally once daily, whereas ICS are often prescribed as two to four times per day and they require procedures that are often not followed correctly.

Several other panel studies have found adverse associations between asthma outcomes and criteria air pollutants among subjects taking anti-inflammatory medications (Delfino et al. 2003; Gent et al. 2003; Lewis et al. 2005; Mortimer et al. 2000; Rabinovitch et al. 2006) whereas other panel studies have shown larger associations among subjects not taking any anti-inflammatory medications (Delfino et al. 1998, 2002; Koenig et al. 2003, 2005; Mar et al. 2005). It is conceivable that subjects not taking anti-inflammatory medications who have persistent asthma are more susceptible than comparable subjects who take sufficient controller medications. Further investigation is required to determine how the source and composition of particle exposures contribute to such differences in group susceptibility.

These issues underlie two important limitations of our study. First, we are reliant on surrogate markers of pollutant components (EC, OC, and NO$_2$) and limited information on sources. Second, the range of individual FEN$_{o}$ overlapped with concentrations seen in healthy individuals, as previously described (Buchvald et al. 2005). This issue is tied to the still unresolved questions about what FEN$_{o}$ levels fully represent, and what the between-individual determinants of FEN$_{o}$ levels are. Other perturbations besides inflammation might increase NO release.

Conclusions

Exhaled NO in schoolchildren with asthma was associated with personal and ambient background exposure to particulate air pollutants and NO$_2$. We found differences in association by medication use, suggesting effect modification and pointing to the importance of assessing individual susceptibility. We also conclude that PM associations may be missed when ambient mass-based methods are used alone, which may not be sufficiently representative of causal pollutant components for some populations or individuals. The relatively weak findings for ambient particle mass compared with EC, OC, and NO$_2$ suggest that protecting public health may be insufficient using only a particle mass-based standard, as is currently the case under the U.S. National Ambient Air Quality Standards. Supplemental measurements of particle composition and ultrafine particles (Sioutas et al. 2005), preferably on an hourly scale, are needed to better assess the health impact of particulate air pollution.

We found that association of FEN$_{o}$ with personal and ambient NO$_2$ was largely independent of personal and ambient EC and OC fractions of PM$_{2.5}$ in two-pollutant models, suggesting that both ambient and personal NO$_2$ represents other causal pollutant components not sufficiently captured by ambient EC or OC in our study regions. In addition, NO$_2$ is a potent oxidant, and experimental data support the hypothesis that oxidative stress may be an important mechanism underlying NO$_2$ toxicity (Persing et al. 2002). To address this gap in knowledge on causal components, continued research is needed on toxic air pollutants expected to have adverse respiratory effects based on biologic mechanisms (e.g., oxidative stress responses). There is also need for health effects studies using source-specific as opposed to a species-specific exposure assessments of air pollution (Ebel et al. 2005). These recommendations are supported by our contrasting results for personal versus ambient air pollution, especially the two-pollutant model showing independence for personal versus ambient EC. The different lag associations ranging from an immediate FEN$_{o}$ response to the preceding 5 hr of PM$_{2.5}$ to 2-day average PM$_{2.5}$ also suggest different sources and varying biologic mechanisms are at play.

In addition to the relevance of the results presented here to acute asthma exacerbations, repeated insults from air pollutants may lead to chronic adverse effects on childhood asthma. There is evidence that persistent airway inflammation in asthmatics leads to airway remodeling, diminished lung growth, and permanent lung function impairment (Fabbri et al. 1998).
REFERENCES

Adamkiewicz G, Ebelt S, Syring M, Stater J, Speizer FE, Schwartz J, et al. 2004. Association between air pollution exposure and exhaled nitric oxide in an elderly population. Thorax 59:204–209.

American Thoracic Society (ATS) and European Respiratory Society (ERS). 2005. ATS/ERS recommendations for stan-
dardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide. 2005. Am J Respir Crit Care Med 171:912–930.

Buchvald F, Baraldi E, Carraro S, Gaston B, De Jongste J, Adamkiewicz G, Ebelt S, Syring M, Slater J, Speizer FE, Schwartz J, Delfino RJ. 2002. Epidemiological evidence for asthma and expo-

Fabbri LM, Caramori G, Beghe, Papi A. 1998. Physiologic conse-

Diggle P, Heagerty P, Liang KY, Zeger S. 2002. Analysis of

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INCEDO Method. J Air Waste Manage Assoc 52:1333–1341.

Gent JF, Triche E, Holford TR, Balanger K, Bracken MB, Beckett WS, et al. 2003. Association of low-level ozone and fine particles with respiratory symptoms in children with asthma. JAMA 290:1859–1867.

Gong H Jr, Linn WS, Terrell SL, Anderson KR, Clark KW. 2001. Anti-inflammatory and lung function effects of montelukast (Singulair) in asthmatic volunteers exposed to sulfur dioxide. Chest 120:492–498.

Hamid Q, Tufik MK, Liu MC, Moobal R. 2003. Inflammatory cells in asthma: mechanisms and implications for therapy. J Allergy Clin Immunol 111(suppl 1):S5–S12.

Hankinson JO, Genencrantz JR, Fedan KB. 1999. Spirometric refer-

dence values from a sample of the general U.S. population. Am J Respir Crit Care Med 159:179–187.

Janssen KL, Larson TV, Koenig JG, Mar TF, Fields C, Stewart J, et al. 2005. Associations between health effects and particu-
late matter and black carbon in subjects with respiratory disease. Environ Health Perspect 113:1741–1746.

Jobbágs Q, Raattsep HC, Hoc WP, de Jongste JC. 2001. Controlled low flow off line sampling of exhaled nitric oxide in children. Thorax 56:285–289.

Jones SL, Kittleton J, Cowan JD, Flannery EM, Hancock CR, McLachlan CR, et al. 2001. The predictive value of exhaled nitric oxide measurements in assessing changes in asthma control. Am J Respir Crit Care Med 164:738–743.

Kim JJ, Smorodinsky S, Lipsert M, Singer BC, Hodgson AT, Ostro B. 2004. Traffic-related air pollution near busy roads: the East Bay Children’s Respiratory Health Study. Am J Respir Crit Care Med 170:520–526.

Koenig JG, Hansen K, Mar TF, Lumley T, Kaufman J, Trenga CA, et al. 2003. Measurement of offline exhaled nitric oxide in a study of community exposure to air pollution. Environ Health Perspect 111:1625–1629.

Koenig JG, Mar TF, Allen RW, Hansen K, Lumley T, Sullivan JH, et al. 2005. Pulmonary effects of indoor- and outdoor-gen-

Kroesbergen A, Jöbsis Q, Raatgeep HC, Hop WC, de Jongste JC. 2001. Controlled low flow off line sampling of exhaled nitric oxide in children. Thorax 56:285–289.

Lipworth BJ. 1999. Leukotriene-receptor antagonists. Lancet 353:57–62.

Liu MC, van Loveren H, et al. 2003. Acute effect of air pollution on respiratory complaints, exhaled NO and biomarkers in nasal lavages of allergic children during the pollen season. Int Arch Allergy Immunol 133:127–137.

Lundell K, Sjöberg BA, Baumann JM, Evans TM. 2005. Bronchoconstriction provoked by exercise in a high-partici-
late-matter environment is attenuated by montelukast. Inhal Toxicol 17:99–105.

McLachlan CR, et al. 2001. The predictive value of exhaled

Meth 157:5196–5198.

Opperhuizen A, Vos JG, et al. 2005. Evaluation of an active personal exposure monitor for NOx, Anal Biocanal Chem 383:955–962.

Opperhuizen A, Vos JG, et al. 2005. Evaluation of an active personal exposure monitor for NOx, Anal Biocanal Chem 383:955–962.

Rabinovitch N, Szefler SJ, Phillips BR, Schatz M, Martinez FD, Zeiger RS, Szefler SJ, Phillips BR, Schatz M, Martinez FD, Opperhuizen A, Vos JG, et al. 2005. Traffic-related air pol-

Zeilinger RM, Kleeman M, Cass GR, Simonetti BRT. 2002. Measurement of emissions from air pollution sources. S. CI-

C32 organic compounds from gasoline-powered motor vehicles. Environ Sci Technol 36:1169–1180.

Schwartz J. 2000. The distributed lag between air pollution and daily death. Epidemiology 11:320–326.

Sheppard L, Slaughter JC, Schildcrout J, Liu LJ, Lumley T. 2005. Exposure and measurement contributions to estimates of acute air pollution effects. J Expo Anal Epidemiol 15:386–378.

Sioutas C, Delfino RJ, Singh M. 2005. Exposure assessment for atmospheric ultrafine particles (UFP) and implications in epi-

demiological research. Environ Health Perspect 113:947–955.

Stauner N, Delfino RJ, Sioutas C, Bufalino C, Fine PM, Meacher D, et al. 2005. Evaluation of an active personal exposure monitor for NOx, Anal Biocanal Chem 383:955–962.

Steerenberg PA, Bischoff EW, de Klerk A, Verlaan AP, Jonploots LM, van Loveren H, et al. 2003. Acute effect of air pollution on respiratory complaints, exhaled NO and biomarkers in nasal lavages of allergic children during the pollen season. Int Arch Allergy Immunol 133:127–137.

Steerenberg PA, Nierkens S, Fischer PH, van Loveren H, Opperhuizen A, Vos JG, et al. 2001. Traffic-related air pol-

Van Amsterdam JG, Verlaan BP, Van Loveren H, Elzakker BG, Vos SG, Opperhuizen A, et al. 1999. Air pollution is associated with increased level of exhaled nitric oxide in nonsmoking healthy subjects. Arch Environ Health 54:321–325.

Verbeke G, Molenberghs G. 2001. Linear Mixed Models for

Wasserman L, Linn WS, Terrell SL, Anderson KR, Clark KW. 2001. Association of low-level ozone and fine particles with respiratory symptoms in children with asthma. JAMA 290:1859–1867.

Zitnick MR, Kleerup EC, Tashkin DP. 2004. Exhaled nitric oxide in the assessment of asthma. Curr Opin Pulm Med 10:31–36.

Zeiger RS, Szefler SJ, Phillips BR, Schatz M, Martinez FD, Chinchilli VM, et al. 2006. Response profiles to fluticasone and montelukast in mild-to-moderate persistent childhood asthma. J Allergy Clin Immunol 117:45–52.