



# The effect of ambient air pollution on exhaled nitric oxide in the Children's Health Study

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**ABSTRACT:** We assessed the effect of daily variations in ambient air pollutants on exhaled nitric oxide fraction ( $F_{eNO}$ ) using data from a cohort of school children with large differences in air pollutant exposures from the Children's Health Study.

Based on a cohort of 2,240 school children from 13 Southern Californian communities, cumulative lagged average regression models were fitted to determine the association between  $F_{eNO}$  and ambient air pollution levels from central site monitors with lags of up to 30 days prior to  $F_{eNO}$  testing.

Daily 24-h cumulative lagged averages of particles with a 50% cut-off aerodynamic diameter of 2.5  $\mu\text{m}$  ( $PM_{2.5}$ ; over 1–8 days) and particles with a 50% cut-off aerodynamic diameter of 10  $\mu\text{m}$  ( $PM_{10}$ ; over 1–7 days), as well as 10:00–18:00 h cumulative lagged average of  $O_3$  (over 1–23 days) were significantly associated with 17.42% ( $p < 0.01$ ), 9.25% ( $p < 0.05$ ) and 14.25% ( $p < 0.01$ ) higher  $F_{eNO}$  levels over the interquartile range of 7.5  $\mu\text{g}\cdot\text{m}^{-3}$ , 12.97  $\mu\text{g}\cdot\text{m}^{-3}$  and 15.42 ppb, respectively. The effects of  $PM_{2.5}$ ,  $PM_{10}$  and  $O_3$  were higher in the warm season. The particulate matter effects were robust to adjustments for effects of  $O_3$  and temperature and did not vary by asthma or allergy status.

In summary, short-term increases in  $PM_{2.5}$ ,  $PM_{10}$  and  $O_3$  were associated with airway inflammation independent of asthma and allergy status, with  $PM_{10}$  effects significantly higher in the warm season.

**KEYWORDS:** Airway inflammation, air pollution, children's respiratory health, environmental epidemiology, exhaled nitric oxide

Several studies have shown that exposure to ambient air pollution at levels below the limits set by current regulatory standards are associated with significant adverse effects on children's respiratory health. Among a spectrum of adverse effects, ambient air pollution has been associated with deficits in lung growth [1, 2], increases in bronchitic symptoms [3] and development and/or exacerbation of asthma [4–7]. These results demonstrated that long-term exposure to ambient air pollution significantly impairs children's respiratory health, potentially leading to irreversible health consequences that may lead to premature morbidity and mortality later in life.

It is important to understand the contribution of air pollutants to the underlying pathophysiological

mechanisms for these observed effects of air pollution in order to optimise interventions and regulatory policies designed to protect the most susceptible children. One mechanism that may play an important role is airway inflammation. In the past, it was not practically feasible to study airway inflammation directly in large population-based prospective studies. The exhaled nitric oxide fraction ( $F_{eNO}$ ) is a promising marker of important aspects of airway inflammation that has been developed and validated in the past decade [8, 9]. Measurement of  $F_{eNO}$  allows the assessment of inflammation in large epidemiological studies designed to identify populations that may be at increased risk for adverse health outcomes [10, 11].

In this study, we assessed the hypothesis that current levels of ambient air pollutants (including

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particles with a 50% cut-off aerodynamic diameter of 2.5 and 10  $\mu\text{m}$  (PM<sub>2.5</sub> and PM<sub>10</sub>, respectively), NO<sub>2</sub> and O<sub>3</sub>) are associated with airway inflammation in children as measured by FeNO. We investigated this hypothesis as part of the Southern California Children's Health Study (CHS), an ongoing prospective cohort study of environmental and genetic determinants of respiratory health in Southern Californian children [7, 12, 13].

## METHODS

### Study design and subjects

Details on the design, site selection, subject recruitment and assessment of health effects are reported elsewhere [7]. Briefly, a cohort of 5,093 children from 13 communities in Southern California was enrolled in 2002–2003 from kindergarten and first grade classrooms (aged 5–7 yrs). At baseline and each subsequent year of follow-up, parents completed a written questionnaire. Informed assent was sought from each child, and informed consent from a parent or guardian. The protocol was approved by the University of Southern California Institutional Review Board.

Measurement of FeNO was performed at schools during the 2004–2005 school year using the offline technique according to the American Thoracic Society guidelines [8, 9]. Details of the FeNO collection and quality-control approaches have been reported previously [14, 15]. In a sub-sample (n=361) for whom both off- and online (50 mL·s<sup>-1</sup> flow) FeNO measurement techniques were used, online FeNO levels were reliably predicted (model adjusted R<sup>2</sup>=0.94) using a statistical model that incorporated offline FeNO, ambient NO and lag time between time of collection and FeNO measurement [14]. In the present analysis, predicted online FeNO data obtained from that model were used. Among the 3,544 children available in year 3 of the study, FeNO was measured in 2,792 subjects. We excluded children who did not have technically acceptable FeNO measurement (n=82), and those from Lake Arrowhead due to lack of measurements of particulate matter (n=209). In addition, we excluded 261 subjects because they reported use of inhaled corticosteroids (n=51) or gave no information on their use of medication (n=210). Therefore, the final analysis data set included 2,240 subjects.

Air pollution data were obtained from central monitoring sites in each study community. In addition to hourly data on the day of the test, data on daily 24-h averages of PM<sub>2.5</sub>, PM<sub>10</sub>, NO<sub>2</sub>, temperature and daily 10:00–18:00 h averages of O<sub>3</sub> were obtained for the test date and each of the 60 days prior to FeNO testing in order to fully investigate the lag structure of the air pollution effects and the impact of temperature on the effects of air pollution. More details on the questionnaire information, collection of FeNO and air pollution data are provided in the supplementary data.

Race/ethnicity, physician diagnosis of asthma, history of respiratory allergy (allergic rhinitis and/or hayfever), asthma medication use during the previous 12 months and exposure to second-hand tobacco smoke (SHS) were based on parental reports. Height and weight were measured on the day of test. Age- and sex-specific percentiles based on the Centers for Disease Control and Prevention body mass index (BMI) growth charts ([www.cdc.gov/NCCDPHP/dnpa/growthcharts/resources/sas](http://www.cdc.gov/NCCDPHP/dnpa/growthcharts/resources/sas).

htm) were used to determine normal, overweight and obesity status of each child.

### Statistical analysis

Descriptive and exploratory data analyses were conducted to: 1) examine the characteristics of the study population; 2) characterise the distribution of the FeNO measurements; and 3) assess the temporal trends of the air pollution levels during the study period. Multiple linear regression, with town-specific intercepts to control for any between community factors including those of regional differences in long-term pollution levels, was used to determine the relationship between levels of FeNO and variations in air pollution levels. A thorough model building process was conducted to examine the lag structure of air pollution effects, with adjustment for potential confounders and effect modifiers. These included age, sex, race/ethnicity, asthma, asthma medication use, history of respiratory allergy, time of FeNO collection, BMI percentiles, SHS, parental education, language of the questionnaire (English/Spanish), season, temperature (minimum, maximum and average levels on test date; and daily average levels for up to 30 days prior to the test), and whether FeNO testing was conducted outdoors. Seasonal effects were assessed by dividing the study period into the "cold" and "warm" seasons. The warm season was defined as March 16, 2005 to June 30, 2005 while the cold season was defined as the period from October 1, 2004 to March 15, 2005 based on Southern California climatic conditions.

Several types of lag-based models were considered, namely: 1) unconstrained lag models; 2) polynomial distributed lag models (PDL); 3) splines-based distributed lag models (SDLM); and 4) cumulative lag models. Linear, quadratic and cubic polynomials forms were considered for the PDL models with lags of up to 30 days prior to FeNO testing. Similarly, SDLM considered linear, quadratic and cubic splines with up to three knots chosen *via* a grid search over the 1–30-day lag period. Model selection was based on Akaike Information Criterion (AIC) since the models were not considered to be nested [16]. After choosing the final models for each pollutant, potential effect modification by sex, asthma, respiratory allergy and season were examined. Two pollutant models, guided by the correlation structure to avoid potential multi-collinearity, were fitted to examine co-dependency of the various pollutants. Sensitivity analysis on the lag structure was conducted using lagged effects of up to 60 days, whenever appropriate, to assess whether the air pollution effects extended beyond the 30-day lags. Similar analyses were also conducted on hourly lags for the 24 h prior to FeNO testing. Effects of long-term pollution levels were investigated as potential confounders or effect modifiers of the short-term effects in models that included random intercepts. All models were fitted to the SAS Version 9.1 statistical package (SAS Institute, Cary, NC, USA) and the R-statistical software. Statistical significance was assessed assuming a 0.05 significance level and a two-sided alternative hypothesis.

## RESULTS

The study population was equally divided between boys and girls with the majority of the participants reporting their ethnicity as Hispanic (55.7%) or non-Hispanic White (34.0%)

(table 1). FeNO levels were significantly higher in Asians, those filling in Spanish language questionnaires, children with asthma or respiratory allergy and those who came from households with less educated parents (less than high school), as reported previously [15]. Slight differences between the populations analysed here and previously [15] relate primarily to

the availability of air pollution data. FeNO levels were not significantly associated with sex, BMI or exposures to tobacco smoke (*in utero* and second hand).

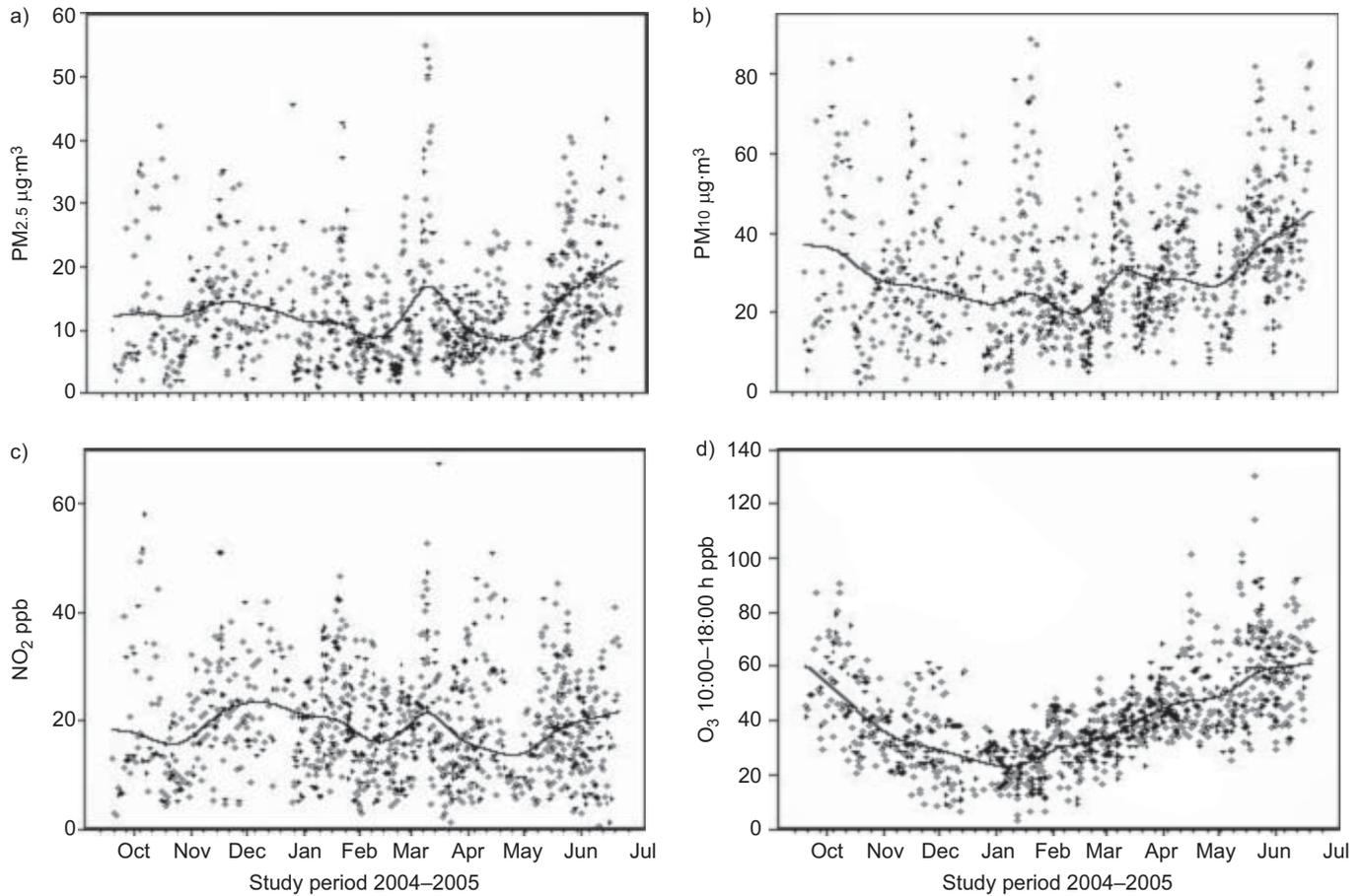
The time trends of daily 24-h average levels of PM<sub>2.5</sub>, PM<sub>10</sub>, NO<sub>2</sub> and daily 10:00–18:00 h average levels of O<sub>3</sub> during the study period (September 19, 2004 to June 22, 2005) are shown in figure 1. The raw daily pollution data and the superimposed smooth plots show varying levels of seasonality among the time-series patterns of the ambient levels of the four air pollutants. Table 2 presents the correlation structure between the four pollutants of interest which were calculated using 24-h averages over a 30-day lag before each subject's FeNO measurement, except ozone, which used averages of 10:00–18:00 h O<sub>3</sub> concentrations over a 30-day lag prior to FeNO testing. The correlations are shown for warm and cold seasons because we observed differences in the cold and warm seasons. PM<sub>10</sub> and PM<sub>2.5</sub> were highly positively correlated in both seasons. O<sub>3</sub> and NO<sub>2</sub> were negatively correlated in the cold season. O<sub>3</sub> was positively correlated with PM<sub>10</sub> and PM<sub>2.5</sub> in the warm season, but was virtually uncorrelated with the particulate matter pollutants during the cold season.

Based on the AIC [16], we chose the best model for each pollutant among polynomial/spline distributed lag models (linear, quadratic or cubic) and cumulative lagged average models with lags up to 30 days prior to FeNO tests. We found that 8, 7, 6 and 23 cumulative lagged-daily average levels were the best models for PM<sub>2.5</sub>, PM<sub>10</sub>, NO<sub>2</sub> and O<sub>3</sub>, respectively. Figure 2 shows the lag structure over a 30-day period of the cumulative averaged air pollution effects from fully adjusted multiple linear regression models. In addition to the pollutant under consideration, the final models adjusted for community, race/ethnicity, sex, asthma, asthma medication use, history of respiratory allergy, age, time of FeNO collection, BMI, SHS, parental education, language of the questionnaire (English/Spanish), season and whether FeNO testing was conducted outdoors. The results from these final models assessing the effects of air pollution on FeNO are presented in table 3 and corresponding partial regression plots depicting the regression lines along with scatter plots of the residuals are presented in the supplementary data (fig. E1). We found that short-term levels of PM<sub>2.5</sub>, PM<sub>10</sub> and O<sub>3</sub> were significantly associated with airway inflammation. Note that these results are interpreted as within-community effects of the air pollution metrics on FeNO because the models included community-specific intercepts [17]. Daily 24-h average PM<sub>2.5</sub> concentration (1–8 day lagged cumulative average) was significantly associated with 17.42% ( $p < 0.01$ ) higher levels of FeNO over the interquartile range (IQR) of 7.5  $\mu\text{g}\cdot\text{m}^{-3}$ . Daily 24-h average PM<sub>10</sub> concentration accumulated over 1–7 day lags was significantly associated ( $p < 0.05$ ) with 9.25% higher FeNO levels per the IQR of 12.97  $\mu\text{g}\cdot\text{m}^{-3}$ . Similarly, 10:00–18:00 h cumulative average O<sub>3</sub> (over 1–23 lag days) concentrations prior to FeNO testing was significantly associated with 14.25% higher FeNO levels over the IQR of 15.42 ppb ( $p < 0.01$ ). There was no statistically significant association with NO<sub>2</sub> (best model selected for 1–6 day lag average), even though tendency towards positive association was observed as in the other three pollutants. There was no significant effect of long-term pollution for any of the four pollutants under consideration (not shown).

**TABLE 1** Characteristics of study population and comparisons of exhaled nitric oxide fraction (FeNO) levels

Variable	Subjects <sup>#</sup>	FeNO <sup>†</sup>	p-value <sup>‡</sup>
<b>Sex</b>			
Girls	1146 (51.16)	11.02 ± 6.61	0.53
Boys	1094 (48.84)	11.25 ± 7.08	
<b>Age yrs</b>			
<8	703 (31.38)	10.8 ± 6.59	0.005
8–9	1102 (49.20)	11.02 ± 6.5	
>9	435 (19.42)	12.18 ± 8.28	
<b>Race/ethnicity</b>			
Non-Hispanic White	828 (37.01)	10.28 ± 5.96	<0.0001
Hispanic	1247 (55.74)	11.59 ± 7.07	
African-American	61 (2.73)	11.82 ± 8.28	
Asian	86 (3.84)	14.15 ± 11.04	
Other	15 (0.67)	10.8 ± 3.89	
<b>Asthma</b>			
Yes	169 (7.54)	13.87 ± 10.41	<0.0001
No	2071 (92.46)	10.91 ± 6.55	
<b>History of respiratory allergy</b>			
Yes	1167 (52.10)	11.94 ± 8	<0.0001
No	1073 (47.90)	10.38 ± 5.61	
<b>Questionnaire language</b>			
Spanish	531 (23.71)	12.3 ± 7.51	<0.0001
English	1709 (76.29)	10.8 ± 6.59	
<b>Body mass index</b>			
Underweight <5th percentile	49 (2.19)	10.8 ± 5.62	0.85
Normal 5th to <85th percentile	1368 (61.29)	11.25 ± 6.97	
Overweight 85th to <95th percentile	395 (17.70)	11.13 ± 7.13	
Obese >95th percentile	420 (18.82)	10.91 ± 6.44	
<b>FeNO collected outside classroom</b>			
Yes	111 (4.96)	14.88 ± 9.82	<0.0001
No	2129 (95.04)	11.02 ± 6.72	
<b>Parental education</b>			
Less than high school	424 (20.06)	12.55 ± 7.78	<0.0001
High school	390 (18.45)	11.13 ± 7.24	
Above high school	1300 (61.49)	10.8 ± 6.48	
<b>Exposure to second-hand smoke</b>			
Yes	102 (4.55)	12.06 ± 7.84	0.20
No	2123 (94.78)	11.13 ± 6.79	
<b>In utero exposure to maternal smoking</b>			
Yes	130 (6.11)	10.28 ± 6.06	0.11
No	1998 (93.89)	11.25 ± 6.97	

Data are presented as n (%) or mean ± SD, unless otherwise stated. <sup>#</sup>: numbers do not always add up because of missing data; <sup>†</sup>: geometric mean and SD obtained from a univariate model testing equality of group means; <sup>‡</sup>: tests for the equality of group means (did not include missing category).



**FIGURE 1.** The time trends of daily 24-h average levels of a) particles with a 50% cut-off aerodynamic diameter of 2.5 µm (PM<sub>2.5</sub>), b) particles with a 50% cut-off aerodynamic diameter of 10 µm (PM<sub>10</sub>), c) NO<sub>2</sub> and d) daily 10:00–18:00 h average levels of O<sub>3</sub> during the study period (September 19, 2004 to June 22, 2005). The superimposed smooth plot depicts the overall temporal trend and is based on a smooth spline function with 10 degrees of freedom.

The effects of the air pollutants did not significantly vary by sex, asthma or respiratory allergy status (table 4). In season-specific models, PM<sub>10</sub>, PM<sub>2.5</sub> and O<sub>3</sub> had relatively larger effects in the warm season, but this seasonal difference was

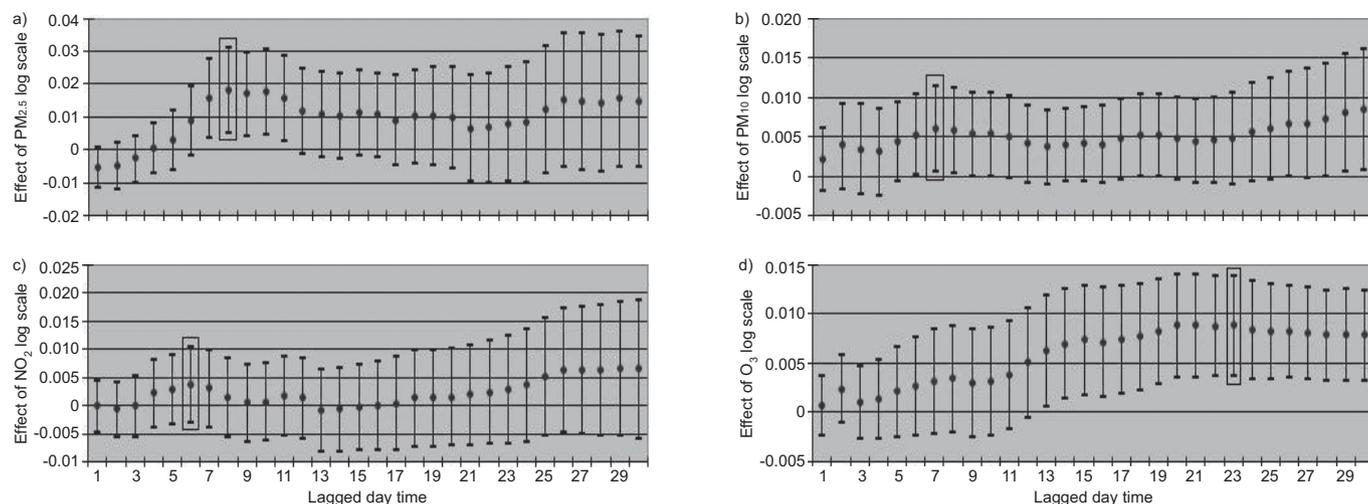
statistically significant for PM<sub>10</sub> only (19.9% versus 4.3% per IQR of 12.97 µg·m<sup>-3</sup>; interaction p=0.02). Although the seasonal differences were not statistically significant, the effects of PM<sub>2.5</sub> and O<sub>3</sub> were also higher in the warm season. In two pollutant models that allowed for co-adjustment of each particulate matter measure with O<sub>3</sub>, the significant effects of PM<sub>2.5</sub> and PM<sub>10</sub> were found to be robust to adjustments for effects of O<sub>3</sub> (not shown).

**TABLE 2** Correlation among air pollutants<sup>#</sup>

Pollutants <sup>†</sup>	PM <sub>2.5</sub>	PM <sub>10</sub>	NO <sub>2</sub>	O <sub>3</sub>
<b>PM<sub>2.5</sub></b>		<b>0.79</b>	<b>0.47</b>	<b>0.61</b>
<b>PM<sub>10</sub></b>	0.72		<b>0.49</b>	<b>0.59</b>
<b>NO<sub>2</sub></b>	0.65	0.55		<b>0.15</b>
<b>O<sub>3</sub></b>	-0.05	0.06	-0.4	

PM<sub>2.5</sub>: particles with a 50% cut-off aerodynamic diameter of 2.5 µm; PM<sub>10</sub>: particles with a 50% cut-off aerodynamic diameter of 10 µm. #: Pearson correlation coefficients (r) between any two pollutants. The data in bold represents correlations during the warm season (March 16, 2005 to June 30, 2005), whereas the un-bolded data shows correlations in the cold season (October 1, 2004 to March 15, 2005). †: correlations are estimated using 24-h averages over a 30-day lag before each subject's exhaled nitric oxide fraction (FeNO) measurement except ozone, which used averages of 10:00–18:00 h ozone concentrations over a 30-day lag prior to FeNO testing.

Several models were fitted to further test the sensitivity of the results to various alternative model specifications. Extending the number of lagged days to 60 days did not alter the final model choices (data not shown). A similar investigation on the hourly lagged effects for the 24 h prior to FeNO testing, based on full considerations for distributed lagged structures, did not reveal any significant findings for any of the pollutants (data not shown). In sensitivity analyses, we found that the pollutant effects were not confounded by levels of temperature (maximum, minimum and average levels) on the day of the test or cumulative 6-day lagged average temperature level (fitted only when there were no issues with multi-collinearity), selected based on a model search similar to that performed for air pollutants (data not shown). The effects of the air pollutants did not significantly vary by long-term pollution levels (dividing the communities into high/low categories at the



**FIGURE 2.** Lag structure over a 30-day period of the cumulative averaged air pollution effects from fully adjusted multiple linear regression models for daily 24-h average levels of a) particles with a 50% cut-off aerodynamic diameter of 2.5  $\mu\text{m}$  (PM<sub>2.5</sub>), b) particles with a 50% cut-off aerodynamic diameter of 10  $\mu\text{m}$  (PM<sub>10</sub>), c) NO<sub>2</sub> and d) daily 10:00–18:00 h average levels of O<sub>3</sub>. The y-axis represents the adjusted effect estimate of each pollutant on natural log transformed exhaled nitric oxide fraction. The best lag for each pollutant, selected *via* Akaike Information Criterion, is indicated by the rectangular box in each panel.

median) or by use of non-inhaled corticosteroid medication (data not shown). Sensitivity analysis conducted to test for finer temporal influences by including terms for months, instead of season, did not give better model performance. Hence, we used the more parsimonious model of adjusting for season in our final models.

## DISCUSSION

We have shown that short-term increases in ambient air pollution are associated with higher levels of FeNO in children, independent of asthma and allergy status. We found that the association with the particulate matter metrics was the strongest, significantly stronger in the warm season for PM<sub>10</sub> and robust to adjustments for effects of other pollutants. Our

data also showed that the effects for PM<sub>2.5</sub> and PM<sub>10</sub> had relatively shorter lag structures compared to those for O<sub>3</sub> that had a longer lag structure of 23 days prior to FeNO measurement.

In this study, our main aim was to evaluate the impact of ambient air pollutants without subjecting the analysis to any *a priori* lags, as no study to our knowledge evaluated these associations in such a manner. We found significant positive associations of PM<sub>2.5</sub>, PM<sub>10</sub>, O<sub>3</sub> and FeNO using larger lags. Our findings are generally consistent with earlier reports [18–26] although we observed longer lagged-effects of ambient air pollutants.

In contrast to other studies conducted in children [18, 21], we did not find significant effects on the shorter hourly time scale during the 24 h prior to FeNO testing. This may be due, in part, to adjustment of ambient NO in our study. From our earlier work which studied the relationship between online and offline FeNO data [14], we reported that association of offline FeNO with ambient NO is likely, in part, to be an artefact from intrusion of high ambient NO concentrations into the offline collection devices and bags [18, 21].

Although there are differences in findings across studies in terms of lagged effects of ambient air pollutants on FeNO in children, there are biologically plausible reasons for such inconsistencies. The differences in findings may reflect differences in levels of exposures with varying degree of correlations among pollutants across geographical regions and seasons [18, 22, 27], testing the hypothesis among susceptible subjects (children with asthma or allergy) [23–27] or assessment in very high exposure environments [20]. For example, exposure to high O<sub>3</sub> concentrations during two summer camp days (8 h average  $\geq 135 \mu\text{g}\cdot\text{m}^{-3}$ ) resulted in a significant increase in FeNO in evenings among healthy children [20]. In another study, DELFINO *et al.* [18] found 2-day moving average of personal PM<sub>2.5</sub> was associated with higher FeNO in children with persistent asthma. These findings suggest that association

**TABLE 3** Results of cumulative daily average lagged air pollutant effect on exhaled nitric oxide fraction (FeNO)<sup>#</sup>

Pollutant <sup>†</sup>	Lag structure	FeNO <sup>‡</sup>
PM <sub>2.5</sub>	1–8 day average	17.42 (6.15–29.89)**
PM <sub>10</sub>	1–7 day average	9.25 (1.58–17.50)*
NO <sub>2</sub>	1–6 day average	5.13 (-2.34–13.17)
O <sub>3</sub>	1–23 day average	14.25 (5.24–24.04)**

Data are presented as % difference (95% CI). PM<sub>2.5</sub>: particles with a 50% cut-off aerodynamic diameter of 2.5  $\mu\text{m}$ ; PM<sub>10</sub>: particles with a 50% cut-off aerodynamic diameter of 10  $\mu\text{m}$ . <sup>#</sup>: all models are adjusted for community, race/ethnicity, sex, asthma, asthma medication use, history of respiratory allergy, age, time of FeNO collection, body mass index, second-hand smoke, parent education, language of the questionnaire (English/Spanish), season and whether FeNO testing was conducted outdoors. <sup>†</sup>: all pollutants are 24-h average, except for ozone (10:00–18:00 h average). <sup>‡</sup>: results are scaled to the interquartile range values of 7.5  $\mu\text{g}\cdot\text{m}^{-3}$  (PM<sub>2.5</sub>), 12.97  $\mu\text{g}\cdot\text{m}^{-3}$  (PM<sub>10</sub>), 10.62 ppb (NO<sub>2</sub>) and 15.42 ppb (O<sub>3</sub>). \*\*:  $p < 0.01$ ; \*:  $p < 0.05$ .

**TABLE 4** Interaction tests on cumulative daily average lagged air pollutant<sup>#</sup> effects on exhaled nitric oxide fraction ( $F_{eNO}$ )<sup>†</sup>

Modifying factors	PM <sub>2.5</sub> <sup>+</sup>	PM <sub>10</sub> <sup>+</sup>	NO <sub>2</sub> <sup>+</sup>	O <sub>3</sub> <sup>+</sup>
<b>Asthma</b>				
No	18.8 (7.3–31.5)**	9.6 (1.9–17.9)*	5.9 (-1.7–14.1)	14.5 (5.3–24.4)**
Yes	5.8 (-9.1–23.2)	2.1 (-11.4–17.5)	-3.6 (-17.7–12.7)	12.6 (-0.7–27.7) <sup>§</sup>
p-interaction	0.07	0.27	0.22	0.77
<b>Respiratory allergy</b>				
No	13.9 (1.9–27.4)*	7.1 (-1.4–16.4)	4.7 (-1.9–11.7)	12.4 (2.7–23.0)*
Yes	19.6 (7.7–32.9)**	10.8 (2.5–19.9)*	7.8 (1.4–14.7)*	15.4 (6.0–25.7)**
p-interaction	0.22	0.32	0.31	0.40
<b>Season</b>				
Warm	18.4 (6.8–31.4)**	19.9 (7.9–33.2)**	4.8 (-5.0–15.6)	15.3 (5.3–26.2)**
Cold	13.0 (-2.6–31.0)	4.3 (-3.9–13.2)	5.4 (-3.4–15.0)	10.2 (-7.5–31.3)
p-interaction	0.49	0.02	0.92	0.65

Data are presented as % difference (95% CI), unless otherwise stated. PM<sub>2.5</sub>: particles with a 50% cut-off aerodynamic diameter of 2.5 µm; PM<sub>10</sub>: particles with a 50% cut-off aerodynamic diameter of 10 µm. <sup>#</sup>: all pollutants are 24-h average, except for ozone (10:00–18:00 h average); <sup>†</sup>: all models are adjusted for community, race/ethnicity, sex, asthma, asthma medication use, history of respiratory allergy, age, time of  $F_{eNO}$  collection, body mass index, second-hand smoke, parent education, language of the questionnaire (English/Spanish), season and whether  $F_{eNO}$  testing was conducted outdoors. In addition, stratum specific effects of pollution are reported for each modifying factor (asthma, history of respiratory allergy or season) by including interaction terms wherever appropriate. <sup>+</sup>: results are scaled to the interquartile range values of 7.5 µg·m<sup>-3</sup> (PM<sub>2.5</sub>), 12.97 µg·m<sup>-3</sup> (PM<sub>10</sub>), 10.62 ppb (NO<sub>2</sub>) and 15.42 ppb (O<sub>3</sub>). <sup>§</sup>: p<0.1; \*: p<0.05; \*\*: p<0.01.

between ambient air pollutants and  $F_{eNO}$  may depend on level of exposure and individual susceptibility to the inflammatory effects of air pollutants.

We did not find significant influence of a number of potential susceptibility factors (*i.e.* asthma and allergy) on the associations between ambient air pollutants and  $F_{eNO}$  suggesting that current levels of ambient pollutants have the potential to increase nitrosative stress in both healthy and susceptible children. While similar effects of air pollution on  $F_{eNO}$  in atopic and non-atopic adults have been reported previously [28], our results are also in contrast to findings from other studies. For example, BARRAZA-VILLAREAL *et al.* [22] report significant effects of 8-h cumulative averages of PM<sub>2.5</sub> and O<sub>3</sub> on  $F_{eNO}$  levels among asthmatics. However, there did not appear to be significant differences with effects in non-asthmatics, which were nonsignificant due to a small sample size (n=50). In our study, we did not find significant effects for any of the pollutants in the 24 h prior to  $F_{eNO}$  testing even when we only considered children with asthma (data not shown). We found a significant heterogeneity of effects of PM<sub>10</sub> on  $F_{eNO}$  by season in Southern California, and this heterogeneity did not seem to be explained by weather as characterised by temperature. Although the magnitude of correlation between PM<sub>10</sub> and PM<sub>2.5</sub> and NO<sub>2</sub> were comparable between seasons, the correlation between PM<sub>10</sub> and O<sub>3</sub> were strikingly different. The positive association between PM<sub>10</sub> and  $F_{eNO}$  in summer remained robust even after adjusting for O<sub>3</sub>. Further research is needed to evaluate whether compositional difference exists in PM<sub>10</sub> by seasons that could account for the observed differential effects.

The advantages of the current study include: 1) evaluation of the air pollution effects on  $F_{eNO}$  using a large, ethnically diverse population of children; 2) substantial range in exposures to the spectrum of complex multi-pollutant mixtures available in the

Southern California region which could represent the full national range; 3) a thorough investigation on the lag structure of up to 60 days prior to  $F_{eNO}$  testing; 4) determination of independent effects of pollutants in a multi-pollutant setting; and 5) testing whether the associations varied by susceptibility factors (asthma and allergy) and by season.

There are some concerns in the scientific community regarding use of  $F_{eNO}$  as a marker of airway inflammation [29, 30]. However, our data, along with a large body of evidence from earlier work, indicates that  $F_{eNO}$  is influenced significantly by ambient air pollution and is correlated with atopic conditions (asthma, allergy, sputum or blood eosinophil and airway hyperresponsiveness), although such correlation is often not strong. Therefore, it can be argued that  $F_{eNO}$  reflects air pollution responsive domains of airway inflammation and more research is needed to understand its correlates.

The results from our study should be interpreted in light of some limitations. The cross sectional nature of the analysis precludes from adequately assessing the independent effects of short-term fluctuations of air pollution on  $F_{eNO}$ , after adjustments for long-term regional effects of air pollution. Our  $F_{eNO}$  data was based on “online” predictions from an offline measure. While this process might potentially introduce misclassification and measurement error, there was a very strong correlation between the two measures (model  $r^2=0.94$ ) [14]. The main study findings were essentially identical when the offline  $F_{eNO}$  values were used in our final models. There is potential for misclassification of exposure assignments because we do not have information on time–activity patterns for the study subjects for the duration of the lags evaluated in the study; however, the regional nature of particulate matter and O<sub>3</sub> reduces the potential misclassification. Further research is warranted that incorporates time–activity pattern to improve exposure assignment.

Our air pollution measures were obtained from central site monitors placed in each of our study communities. To minimise the confounding of location and seasonal effects, each community was visited at least twice in different seasons. However, there is still potential for residual spatio-temporal confounding since the study design and budgetary/logistical limitations did not allow measurement of FeNO levels in all communities across the entire study period. To the extent possible, our models included seasonal and other temporal factors as adjustment factors and season-specific models were considered. Controlling for temporal effects using month specific factors and/or smooth functions of time did not result in better model fit compared to those adjusted for season in our final models. Missing days of exposure and interpolation of air pollution data to fill in data gaps with modelled predictions using data from nearby monitors are potential sources of bias. However, sensitivity analyses that limited use to complete data did not alter the main study findings (data not shown).

Our ability to thoroughly assess the effects of use of asthma and allergy medication is limited. We used parent-reported questionnaire based information to collect information on history of respiratory allergy, physician diagnosis of asthma, and medication use for asthma in order to assess the effects of these important factors on confounding and/or modifying the association between air pollution and FeNO. Our findings are internally consistent showing higher FeNO among children with asthma or respiratory allergy.

In conclusion, our data showed that current levels of ambient air pollutants (often meeting regulatory standards) are associated with increased FeNO in children with and without asthma. Because FeNO measurement allows evaluation of airway inflammation noninvasively, use of this biomarker is useful in population-based epidemiological research in assessing the impact of ambient air pollutants on nitrosative stress in airways. We recently reported that elevated FeNO is associated with increased risk of new onset asthma [31], a finding supported by a recent study that found FeNO could predict wheeze onset in adults [11]. Further research is warranted to examine whether FeNO could predict asthma/wheeze incidence in children exposed to different levels of ambient air pollution. If such association exists, then measurement of FeNO could be used as a useful intermediate marker to detect susceptible children who are at high risk of adverse respiratory health outcomes from air pollution.

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#### STATEMENT OF INTEREST

A statement of interest for F. Lurman can be found at [www.ersjournals.com/site/misc/statements.xhtml](http://www.ersjournals.com/site/misc/statements.xhtml)

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