



Early View

Research letter

Long-term exposures to air pollutants affect FeNO in children: a longitudinal study

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Title: Long-term Exposures to Air Pollutants Affect FeNO in Children: A Longitudinal Study

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Key messages

- We show strong evidence that long-term exposures to air pollutants affect FeNO, independent of the well-documented associations with short-term exposures to air pollution.
- Longitudinal FeNO measurements may be useful as an early marker of chronic respiratory effects of long-term air pollution exposures in children.

Capsule summary

Annual average PM_{2.5} and NO₂ were associated with airway inflammation as measured by FeNO in schoolchildren, adding new evidence that long-term exposure affects FeNO beyond the well-documented short-term effects.

Key words:

FeNO, pollution, particulate matter, nitrogen dioxide, children

Abbreviations

ATS: American Thoracic Society

CHS: Southern California Children's Health study

FeNO: Fractional exhaled nitric oxide

NO₂: Nitrogen dioxide

O₃: Ozone

PM_{2.5}: particulate matter with an aerodynamic diameter of 2.5 µm or less

PM₁₀: particulate matter with an aerodynamic diameter of 10 µm or less

To the Editors

Fractional exhaled nitric oxide (FeNO) is a marker of airway inflammation shown to be responsive to short-term air pollution exposures[1-4]. Although a number of cross-sectional studies have related FeNO to either location-based proxies for long-term exposures to traffic/industrial activity or long-term (seasonal to annual average) ambient pollution[5-9], there is lack of longitudinal study and the effects of longer-term air pollution exposures on FeNO are still not well studied, especially for school children. To fill the knowledge gap, we conducted a new study on longitudinal assessments of FeNO to air pollutant exposures in the Southern California Children's Health Study (CHS) to determine whether FeNO is a marker for chronic effects of air pollution exposures after accounting for short-term exposures.

Participants were part of the most recent CHS cohort, recruited from kindergarten and first grade classrooms in the 2002/03 school year. Detailed information on study design, participant recruitment, and data collection has been reported previously.[10] This analysis includes 3607 school children from 12 communities in Southern California, who had FeNO assessed up to 6 times in approximately annual visits from 2004/05 through 2011/12. FeNO was measured at schools following the American Thoracic Society (ATS) guidelines using an offline technique at the first two visits and online technique in the subsequent four visits. For offline measurement, breath was collected in aluminized Mylar bags using commercial breath sampling kits (Sievers Division, GE Analytical Instruments, Boulder, Colorado, USA). For online measurement, FeNO was measured directly using EcoMedics CLD-88-SP analyzers with DeNOx accessories to provide NO-free inhaled air (EcoPhysics Inc, Ann Arbor, MI, USA/Duernten, Switzerland). Offline FeNO data were converted to estimates of online FeNO at a 50 ml/sec expiratory flow. Details of FeNO data collection, quality control, and conversion procedures have been reported previously.[11-13]

Daily 24-hour average PM_{2.5}, PM₁₀, NO₂, O₃ (10am-6pm only), and temperature data were obtained for the study period from central monitoring sites in each community operated by local air pollution agencies. For each pollutant, community-specific study period averages were computed (an eight-year average, from 2004/05-2011/12). Annual average pollution concentrations were calculated based on exposure data in the 12 months preceding the FeNO test date in that participant's community. Weekly averages were calculated based on the 7 days preceding the FeNO test date. The primary "long-term" exposure of interest for this study was the annual within-community fluctuation, defined as the difference between annual averages and the eight-year average concentration for each community. We refer to weekly averages as "short-term" exposures hereafter.

FeNO had a right-skewed distribution, so we performed all analyses with natural log-transformed FeNO. We summarized the characteristics of the study participants, and compared the distribution of baseline year log FeNO by these characteristics. We employed linear mixed effect models to examine the association of log FeNO with annual within-community fluctuations in air pollution, adjusting for short-term exposures, confounders, and design variables as well as log FeNO at the previous visit. Potential confounders included sex, race/ethnicity, and respiratory allergy at baseline, as well as time-varying measures of asthma status, asthma medication use, current wheeze, secondhand tobacco smoke exposure, recent respiratory illness, concurrent room air nitric oxide, month of FeNO test, and average

temperature in the week preceding the FeNO test. We also evaluated whether long-term exposure associations were modified by: baseline asthma, baseline allergy, sex, and ethnicity.

The 3607 children, with mean age 9 at baseline, were evenly distributed by sex (male: 49.7%), primarily Hispanic (56.3%) or non-Hispanic White (33.2%), 41.5% had allergies, 15.1% had asthma, and 3% had reported a recent respiratory illness. At baseline, the geometric mean FeNO was 12.1 ppb (geometric SD: 1.9 ppb) and baseline FeNO was statistically significantly associated with most participant characteristics. Specifically, boys on average had significantly higher FeNO than girls (p-value=0.015). FeNO differed by race/ethnicity (p-value <0.001), with African American children having higher FeNO than the other race/ethnicity groups. Children with respiratory conditions, such as asthma, wheeze, allergy, and recent respiratory illness, had statistically significantly higher FeNO than those without (p-values all <0.001). FeNO was positively associated with age and height (p-value <0.001), but not BMI (p-value=0.831). FeNO varied by month of collection (p-value<0.001), with the lowest geometric means in cooler months (December – March).

We found that long-term exposures to PM_{2.5} and NO₂ were positively associated with within-person changes in FeNO, after adjustment for covariates and short-term exposure (Table 1). A one SD (2.0 µg/m³) increase in the annual within-community fluctuation of PM_{2.5} was associated with a 4.6% within-participant increase in FeNO (95% CI: 2.3%-6.8%), controlling for covariates including FeNO at the previous visit and seven-day average PM_{2.5}. Similarly, a one SD (2.7 ppb) increase in the annual within-community fluctuation of NO₂ was associated with a 6.5% within-participant increase in FeNO (95% CI: 4.1%-8.9%), controlling for covariates. We found no evidence for an association of long-term PM₁₀ and FeNO. Long-term O₃ was negatively associated with FeNO in the primary analysis, but this association was attenuated and no longer statistically significant in sensitivity analyses. There was evidence that the associations of long-term PM_{2.5} and NO₂ with FeNO varied by sex, with larger estimated associations in females (interaction p-values: 0.007 for PM_{2.5} and 0.066 for NO₂). We still observed statistically significant positive associations of long-term PM_{2.5} and NO₂ with FeNO after: (a) removing the five communities followed only through four years, (b) removing one community at a time, and (c) taking out the adjustment for short-term air pollution.

This study provides a strong evidence supporting the hypothesis that long-term exposures to air pollutants affect FeNO, independent of the well-documented associations with short-term exposure to air pollution. Results of this longitudinal study (using all six FeNO visits in the CHS) are consistent with a preliminary longitudinal analysis of CHS data (using only the first two online FeNO assessments),[14] which had found *within-participant* associations of long-term PM_{2.5} and NO₂ (but not PM₁₀ or O₃) with FeNO while adjusting for short-term exposure. The previous preliminary CHS study had targeted within-participant changes in FeNO using a different but complementary statistical approach in which the change in FeNO was modeled as a function of the change in annual average air pollution.[14] When studying FeNO, it is important to consider within-participant changes over time from longitudinally collected data, given the considerable unexplained across-participant heterogeneity in FeNO typically observed in cross-sectional studies (e.g., R²<0.3).[15] Indeed, in our data we observed moderately strong autocorrelation in FeNO indicating relatively stable within-person FeNO. Many study communities observed declines in annual average PM_{2.5} and NO₂ during the study period. These

declines were a key source of the variation in our long-term exposure metric (within-community annual fluctuations, calculated as the difference between annual averages and the eight-year average concentration for each community). More detailed analytical results and thorough discussion on research strengths and limitations were provided in the full study report, which could be found in the following link

(<https://www.medrxiv.org/content/10.1101/2021.03.01.21252712v1>) or by contacting the corresponding author.

In conclusion, our findings provide the strongest evidence to date that long-term PM_{2.5} and NO₂ exposures affect within-participant FeNO, independent of the effects of short-term exposures, even during a study period with declining pollution levels. Longitudinal FeNO measurements may be useful as an early marker of chronic respiratory effects of long-term air pollution exposures in children.

Table 1: Adjusted* percent difference in FeNO associated with a one standard deviation (SD)† increase in long-term air pollution exposures‡

Pollutant	Percent difference in FeNO (95% CI)
PM _{2.5}	4.55 (2.33, 6.82)
PM ₁₀	0.63 (-1.88, 3.21)
NO ₂	6.46 (4.08, 8.9)
O ₃	-2.62 (-4.49, -0.71)

*Models adjusted for: sex, race, respiratory allergy, asthma, medication use, wheeze, secondhand tobacco smoking, recent respiratory illness, room air nitric oxide, month, temperature, prior 7 day average of the same pollutant, and natural log-transformed FeNO at the previous visit.

†Long-term exposures are annual within-community fluctuations, calculated as the difference between annual air pollutant concentration and eight-year average concentration.

‡SDs are: 2.0 µg/m³ for PM_{2.5}, 6.7 µg/m³ for PM₁₀, 2.7 ppb for NO₂ and 2.5 ppb for O₃.

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