Long-term exposures to air pollutants affect F_{eNO} in children: a longitudinal study

Received: 4 Jan 2021
Accepted: 5 Aug 2021

To the Editors:

Fractional exhaled nitric oxide (F_{eNO}) 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 F_{eNO} 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 F_{eNO} are still not well studied, especially for school children. To fill the knowledge gap, we conducted a new study on longitudinal assessments of F_{eNO} to air pollutant exposures in the Southern California Children's Health Study (CHS) to determine whether F_{eNO} 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 2002–2003 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 F_{eNO} assessed up to six times in approximately annual visits from 2004–2005 through 2011–2012. F_{eNO} was measured at schools following the American Thoracic Society 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 aluminised Mylar bags using commercial breath sampling kits (Sievers Division, GE Analytical Instruments, Boulder, CO, USA). For online measurement, F_{eNO} was measured directly using EcoMedics CLD-88-SP analysers with DeNOx accessories to provide NO-free inhaled air (EcoPhysics Inc., Ann Arbor, MI, USA/Duernten, Switzerland). Offline F_{eNO} data were converted to estimates of online F_{eNO} at a 50 $mL \cdot s^{-1}$ expiratory flow. Details of F_{eNO} data collection, quality control and conversion procedures have been reported previously [11–13].

Daily 24-h average $PM_{2.5}$, PM_{10} (particles with a 50% cut-off aerodynamic diameter of 2.5 or 10 µm), NO_2, O_3 (10:00–18:00 h 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 8-year average, from 2004–2005 to 2011–2012). Annual average pollution concentrations were calculated based on exposure data in the 12 months preceding the F_{eNO} test date in that participant's community. Weekly averages were calculated based on the 7 days preceding the F_{eNO} 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 8-year average concentration for each community. We refer to weekly averages as "short-term" exposures hereafter.

F_{eNO} had a right-skewed distribution, so we performed all analyses with natural log-transformed F_{eNO}. We summarised the characteristics of the study participants, and compared the distribution of baseline year log F_{eNO} by these characteristics. We employed linear mixed effect models to examine the association of log F_{eNO} with annual within-community fluctuations in air pollution, adjusting for short-term exposures, confounders and design variables, as well as log F_{eNO} 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, second-hand tobacco smoke exposure, recent respiratory illness, concurrent room air nitric oxide, month of F_{eNO} test and average temperature in the week preceding the

Shareable abstract (@ERSpublications)
Annual average $PM_{2.5}$ and NO_2 were associated with airway inflammation as measured by F_{eNO} in schoolchildren, adding new evidence that long-term exposure affects F_{eNO} beyond the well-documented short-term effects https://bit.ly/3CGfYXN

Cite this article as: Zhang Y, Eckel SP, Berhane K, *et al*. Long-term exposures to air pollutants affect F_{eNO} in children: a longitudinal study. *Eur Respir J* 2021; 58: 2100705 [DOI: 10.1183/13993003.00705-2021].

F_{eNO} 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 years 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 F_{eNO} was 12.1 ppb (geometric SD 1.9 ppb) and baseline F_{eNO} was statistically significantly associated with most participant characteristics. Specifically, boys on average had significantly higher F_{eNO} than girls (p-value=0.015). F_{eNO} differed by race/ethnicity (p <0.001), with African-American children having higher F_{eNO} than the other race/ethnicity groups. Children with respiratory conditions, such as asthma, wheeze, allergy and recent respiratory illness, had statistically significantly higher F_{eNO} than those without (p-values all <0.001). F_{eNO} was positively associated with age and height (p<0.001), but not body mass index (p=0.831). F_{eNO} varied by month of collection (p-value<0.001), with the lowest geometric means in cooler months (December to March).

We found that long-term exposures to $PM_{2.5}$ and NO_2 were positively associated with within-person changes in F_{eNO}, after adjustment for covariates and short-term exposure (table 1). A one standard deviation (2.0 $\mu g \cdot m^{-3}$) increase in the annual within-community fluctuation of $PM_{2.5}$ was associated with a 4.6% within-participant increase in F_{eNO} (95% CI 2.3–6.8%), controlling for covariates including F_{eNO} at the previous visit and 7-day average $PM_{2.5}$. Similarly, a one standard deviation (2.7 ppb) increase in the annual within-community fluctuation of NO_2 was associated with a 6.5% within-participant increase in F_{eNO} (95% CI 4.1–8.9%), controlling for covariates. We found no evidence for an association of long-term PM_{10} and F_{eNO}. Long-term O_3 was negatively associated with F_{eNO} 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 NO_2 with F_{eNO} varied by sex, with larger estimated associations in females (interaction p-values: 0.007 for $PM_{2.5}$ and 0.066 for NO_2). We still observed statistically significant positive associations of long-term $PM_{2.5}$ and NO_2 with F_{eNO} after: 1) removing the five communities followed only through 4 years; 2) removing one community at a time; and 3) taking out the adjustment for short-term air pollution.

This study provides strong evidence supporting the hypothesis that long-term exposures to air pollutants affect F_{eNO}, independent of the well-documented associations with short-term exposure to air pollution. Results of this longitudinal study (using all six F_{eNO} visits in the CHS) are consistent with a preliminary longitudinal analysis of CHS data (using only the first two online F_{eNO} assessments) [14], which had found within-participant associations of long-term $PM_{2.5}$ and NO_2 (but not PM_{10} or O_3) with F_{eNO} while adjusting for short-term exposure. The previous preliminary CHS study had targeted within-participant changes in F_{eNO} using a different but complementary statistical approach in which the change in F_{eNO} was modelled as a function of the change in annual average air pollution [14]. When studying F_{eNO}, it is important to consider within-participant changes over time from longitudinally collected data, given the considerable unexplained across-participant heterogeneity in F_{eNO} typically observed in cross-sectional studies (*e.g.* R^2<0.3) [15]. Indeed, in our data we observed moderately strong autocorrelation in F_{eNO} indicating relatively stable within-person F_{eNO}. Many study communities observed declines in annual average $PM_{2.5}$ and NO_2 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 8-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

TABLE 1 Adjusted percent difference in fractional exhaled nitric oxide (F_{eNO}) associated with a one standard deviation increase in long-term air pollution exposures

Pollutant	Percent difference in F_{eNO} (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, second-hand tobacco smoking, recent respiratory illness, room air nitric oxide, month, temperature, prior 7 day average of the same pollutant, and natural log-transformed F_{eNO} at the previous visit. Long-term exposures are annual within-community fluctuations, calculated as the difference between annual air pollutant concentration and 8-year average concentration. Standard deviations are: 2.0 $\mu g \cdot m^{-3}$ for particles with a 50% cut-off aerodynamic diameter of 2.5 μm ($PM_{2.5}$); 6.7 $\mu g \cdot m^{-3}$ for PM_{10}; 2.7 ppb for NO_2; and 2.5 ppb for O_3.

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 NO_2 exposures affect within-participant F_{eNO}, independent of the effects of short-term exposures, even during a study period with declining pollution levels. Longitudinal F_{eNO} measurements may be useful as an early marker of chronic respiratory effects of long-term air pollution exposures in children.

Yue Zhang[1,2,3], Sandrah P. Eckel[4], Kiros Berhane[5], Erika Garcia[4], Patrick Muchmore[6], Noa Ben-Ari Molshatzki[4], Edward B. Rappaport[4], William S. Linn[4], Rima Habre[4] and Frank D. Gilliland[4]

[1]Dept of Internal Medicine, University of Utah, Salt Lake City, UT, USA. [2]Dept of Family and Preventive Medicine, University of Utah, Salt Lake City, UT, USA. [3]Veteran Affairs Salt Lake City Health Care System, Salt Lake City, UT, USA. [4]Dept of Preventive Medicine, University of Southern California, Los Angeles, CA, USA. [5]Dept of Biostatistics, Columbia University, New York, NY, USA. [6]CodoniX, Inc., Potomac, MD, USA.

Corresponding author: Yue Zhang (zhang.yue@hsc.utah.edu)

Support statement: This work was supported by the National Institute of Health, Environmental Protection Agency, and Hastings Foundation. Funding information for this article has been deposited with the Crossref Funder Registry.

Conflict of interest: None declared.

References

1 La Grutta S, Ferrante G, Malizia V, *et al.* Environmental effects on fractional exhaled nitric oxide in allergic children. *J Allergy (Cairo)* 2012; 2012: 916926.

2 Scarpa MC, Kulkarni N, Maestrelli P. The role of non-invasive biomarkers in detecting acute respiratory effects of traffic-related air pollution. *Clin Exp Allergy* 2014; 44: 1100–1118.

3 Huang W, Wang G, Lu SE, *et al.* Inflammatory and oxidative stress responses of healthy young adults to changes in air quality during the Beijing Olympics. *Am J Respir Crit Care Med* 2012; 186: 1150–1159.

4 Berhane K, Zhang Y, Linn WS, *et al.* The effect of ambient air pollution on exhaled nitric oxide in the Children's Health Study. *Eur Respir J* 2011; 37: 1029–1036.

5 Cakmak S, Mahmud M, Grgicak-Mannion A, *et al.* The influence of neighborhood traffic density on the respiratory health of elementary schoolchildren. *Environ Int* 2012; 39: 128–132.

6 Dales R, Wheeler A, Mahmud M, *et al.* The influence of living near roadways on spirometry and exhaled nitric oxide in elementary schoolchildren. *Environ Health Perspect* 2008; 116: 1423–1427.

7 Eckel SP, Berhane K, Salam MT, *et al.* Residential traffic-related pollution exposures and exhaled nitric oxide in the children's health study. *Environ Health Perspect* 2011; 119: 1472–1477.

8 Latzin P, Röösli M, Huss A, *et al.* Air pollution during pregnancy and lung function in newborns: a birth cohort study. *Eur Respir J* 2009; 33: 594–603.

9 Liu C, Flexeder C, Fuertes E, *et al.* Effects of air pollution on exhaled nitric oxide in children: results from the GINIplus and LISAplus studies. *Int J Hyg Environ Health* 2014; 217: 483–491.

10 McConnell R, Berhane K, Yao L, *et al.* Traffic, susceptibility, and childhood asthma. *Environ Health Perspect* 2006; 114: 766–772.

11 Linn WS, Rappaport EB, Berhane KT, *et al.* Extended exhaled nitric oxide analysis in field surveys of schoolchildren: a pilot test. *Pediatr Pulmonol* 2009; 44: 1033–1042.

12 Linn WS, Rappaport EB, Eckel SP, *et al.* Multiple-flow exhaled nitric oxide, allergy, and asthma in a population of older children. *Pediatr Pulmonol* 2013; 48: 885–896.

13 Islam T, Urman R, Gauderman WJ, *et al.* Parental stress increases the detrimental effect of traffic exposure on children's lung function. *Am J Respir Crit Care Med* 2011; 184: 822–827.

14 Berhane K, Zhang Y, Salam MT, *et al.* Longitudinal effects of air pollution on exhaled nitric oxide: the Children's Health Study. *Occup Environ Med* 2014; 71: 507–513.

15 Garcia-Marcos P, Soriano-Pérez M, Perez-Fernández V, *et al.* Exhaled nitric oxide in school children: searching for the lost variability. *Allergol Immunopathol* 2016; 44: 206–213.