A randomized, double-blind, crossover intervention study of traffic-related air pollution and airway inflammation in healthy adults

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Background: Traffic-related air pollution (TRAP) may increase the risk of respiratory disease. The components of TRAP that are responsible for its respiratory toxicity are largely unknown. The objective was to identify the component(s) of TRAP that cause airways inflammation using fractional exhaled nitric oxide (FE_{NO}) and randomized interventions.

Methods: A randomized, double-blind, crossover intervention study was conducted in which 39 healthy university students spent 2 hours next to a busy road. During exposure, participants wore either a powered air-purifying respirator (PAPR) or an N95 facemask. PAPRs were fitted with a fine particle ($PM_{2.5}$) filter, a $PM_{2.5}$ and volatile organic carbon (VOC) filter, or a sham filter, and were blinded to filter type. The four interventions (three PAPR filters and N95) were assigned randomly for each participant and separated by at least 1 week. FE_{NO} was measured before and immediately after each roadside exposure, and at 1, 2, 4, and 6 hours after exposure.

Results: With the sham PAPR filter, the mean postexposure FE_{NO} increased an average of 2.3 ppb (±4.4) compared with the pre-exposure level. Similar increases in FE_{NO} were seen with both the $PM_{2.5}$ PAPR filter and the N95 mask, but no increase was seen with the combination $PM_{2.5}$ and VOC PAPR filter.

Conclusions: Because PAPR filters do not filter inorganic gases (e.g., NO_2 or carbon monoxide), it is concluded that the VOC component of TRAP rather than either the particulate matter or the inorganic gases component is responsible for the airway inflammation caused by TRAP exposure.

Introduction

Traffic-related air pollution (TRAP) is a particulate and gaseous mixture of vehicle exhaust and other vehicle emissions, as well as road dust that includes particles from brake and tire wear. Vehicular emissions are largely emitted directly, but some volatile organic compounds (VOCs) can be rapidly formed secondarily.[1] Exposure to TRAP has been associated with multiple adverse health effects, including effects on the respiratory and cardiovascular systems.[1] TRAP exposure has also been specifically associated with measures of airway inflammation in both healthy and potentially susceptible populations.[2–5] It is not known whether some specific component(s) of TRAP is/are responsible for these reported effects, and if so, what the component(s) might be. An understanding of the more toxic components in TRAP could point to more focused approaches to reducing the health impacts of TRAP exposure.

Observational studies and human clinical studies using vehicle exhaust exposure chambers have not provided compelling evidence as to the specific components of TRAP of most concern, partly because the components are highly correlated. Animal toxicology studies have similar limitations, although one at least has attempted to implicate specific components,[6] but with the inherent uncertainty of relevance to human settings. Intervention studies employing respirators have the potential to not only experimentally determine the effect of TRAP exposure but, depending on the respirator, to also identify the component(s) of the TRAP mixture responsible for any observed effects. Powered air-purifying respirators (PAPRs) have applications largely in the occupational setting and can be equipped with various filters to remove specific components from the inhaled air. PAPRs employing active and sham filters have been used in investigating in-vehicle roadway particle exposure effects on respiratory and cardiovascular endpoints.[7] Here, we report findings from a randomized, double-blind, crossover study employing a respirator intervention to attempt to identify the component(s) of the TRAP mixture that might be responsible for any observed effect of TRAP exposure on airways inflammation, as measured by fractional exhaled nitric oxide (FE_{NO}).

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Methods

Study sample and design

Thirty-nine healthy university students were recruited from a university in Tianjin, China, using advertisements and word of mouth. A questionnaire was administered by research staff to gather demographic information and information on illness history and current symptoms. All participants declared that they had no history of tobacco smoking (never smokers) or dependence on alcohol, no clinically diagnosed chronic respiratory diseases, and no use of medications. The research protocol was approved by the relevant institutional review board (Medical

Ethics Committee of Tianjin Medical University), and participants gave written informed consent.

Tianjin is a mega-city in China with a population of ~15 million located 130 km southeast of Beijing. The site of the study was an 8-lane thoroughfare in Tianjin. Traffic volume on the roadway was 5,144 passenger vehicles per hour in the morning rush hour, including ~200 buses. No heavy-duty trucks were allowed on the roadway during this time period.

The PAPR (OptimAir3000A, Cranberry Township, MSA, PA) has a filter canister allowing one or a combination of filters to selectively filter out air contaminants. The four intervention modes were: (1) Sham mode (PAPR with no filter in the canister); (2) particulate matter (PM) mode (PAPR with only the particle filter #9920261); (3) PMG mode (PAPR with both the particle and organic gas filters #9920263; these PAPR filters do not filter out inorganic gases such as the nitrogen oxides); and (4) N95 mode (8210 N95 Respirator, 3M Science, St. Paul, MN).

Two study participants arrived at the examination office each day at 6:30 AM before having had breakfast. An FE_{NO} (see below) measurement was obtained. After having a breakfast in the office, participants underwent spirometry, were outfitted with an automated blood pressure cuff for blood pressure measurements every 15 minutes throughout the several hours, and had a 12-lead Holter monitor placed for continuous recording of heart rate and rhythm. Only the FE_{NO} findings are reported here. A research assistant tossed a die to randomly determine the sequence of each participant's intervention mode (sham filter, PM filter, PM + gas filter, N95 mask). Study participants and research assistants carrying out FE_{NO} measurements were blinded to the PAPR filter mode, but not to the N95 mask. The two participants wore the respirator while being escorted from the examination office to the roadside (<500 m in distance) and spent 2 hours during the early morning rush hour (7:30–9:30 AM) sitting 20 m from the edge of the pavement, or intermittently walking leisurely in close proximity. Following exposure, they were immediately escorted back to the examination office where the respirator was removed. Participants remained in the office until 4 PM while they underwent sequential measurements of the health endpoints. Each participant underwent a total of four exposure sessions, one for each of the four intervention modes, with at least a 1-week time interval between sessions.

FE_{NO} measurements

FE_{NO} was measured at baseline in the examination office, then immediately after returning to the office, and then at 1, 2, 4, and 6 hours after returning. FE_{NO} was measured using a portable NIOX MINO (Aerocrine AB, Solna, Sweden) according to standardized procedures recommended by the American Thoracic Society and European Respiratory Society.[8] Briefly, participants were seated without noseclips, breathed in to total lung capacity, and then immediately exhaled for up to 10 seconds while attempting to maintain a flow rate of 50 ml/min. The first acceptable maneuver was used for the analysis.

Roadside air pollution concentration measurements

Real-time measurements were made at the roadside of traffic-related air pollutant ($PM_{2.5}$, NO_2, and black carbon [BC]) concentrations and meteorology (temperature and relative humidity). Table S1; http://links.lww.com/EE/A58, details the instrumentation used for these measurements. All monitoring instruments were placed and fixed in a cart that accompanied the participants during the entire examination period.

Statistical methods

The primary endpoint was change in FE_{NO} from baseline (ΔFE_{NO}). Linear mixed effects models were used to estimate the

Table 1
Characteristics of Study Participants

Characteristic		Number/Mean	Percent/Range
Gender	Male	17	47.2%
	Female	19	52.8%
Age (y)	/	22.7	20–29
Body mass index	/	20.9	15.6–29.4
Height (m)	/	1.68	1.53–1.83
Weight (kg)	/	59	40–90
Education level	Undergraduate	20	55.6%
	Graduate and above	16	44.4%

effect of intervention mode. Models included a random effect of study participant (i.e., random intercept) and indicator variables as fixed effects for the PM, PMG, and N95 modes, with the sham filter as the reference group. Additional models also included fixed effects for linear specifications of the pollutant and meteorological variables, and fixed effects for sex and body mass index. Our primary analysis used the average of all postexposure FE_{NO} values to calculate change from baseline. Additional analyses included the difference from baseline at all postexposure times in the same model rather than the average, with additional indicator variables for time of follow-up measurement (i.e., 0, 1, 2, 4, 6 h). Models were also fit using absolute FE_{NO} values, again with indicator variables for time of each follow-up measurement. A sensitivity analysis was performed in which all participants with baseline FE_{NO} values with more than a 10-ppb difference between any two baseline values were excluded from the analysis.

One-way ANOVA was used to assess differences in air pollutant concentrations and meteorology across intervention modes and for initial assessment of mean FE_{NO} and ΔFE_{NO} across the study time points. A P-value <0.05 was considered statistically significant.

Results

Study participants

Characteristics of the study participants are shown Table 1. All participants were nonsmokers and declared that they had no chronic respiratory symptoms or clinically diagnosed chronic respiratory diseases. Three subjects were excluded because their FE_{NO} was >50 ppb.[9] There were approximately an equal number of men and women. All reported staying in the vicinity of the university within 24 hours of the test day, and none reported a respiratory or other illness on the test day.

FE_{NO} by intervention mode

Mean FE_{NO} across the four intervention modes over the study period (from baseline to 6 hours following roadway exposure) is shown in Figure 1A, and in Table S2; http://links.lww.com/EE/A58, and Figure S2; http://links.lww.com/EE/A58, in the Appendix; http://links.lww.com/EE/A58. In Figure 1A, for the sham intervention, FE_{NO} was not different from baseline immediately after exposure, but rose at 1 hour and continued to be increased for the duration of the 6-hour follow-up period. FE_{NO} was highest at 4 hours following exposure. FE_{NO} was slightly higher at baseline before exposure for the PMG and N95 interventions. Following exposure, FE_{NO} was nevertheless slightly lower in the PMG intervention mode than in the other three intervention modes at 1, 4, and 6 hours following exposure, and also at 2 hours following exposure relative to the sham mode. There were no statistically significant differences in FE_{NO} across the intervention modes at any individual time point by ANOVA (Table S2; http://links.lww.com/EE/A58), as would be expected given the large between-subject variation in FE_{NO} levels.
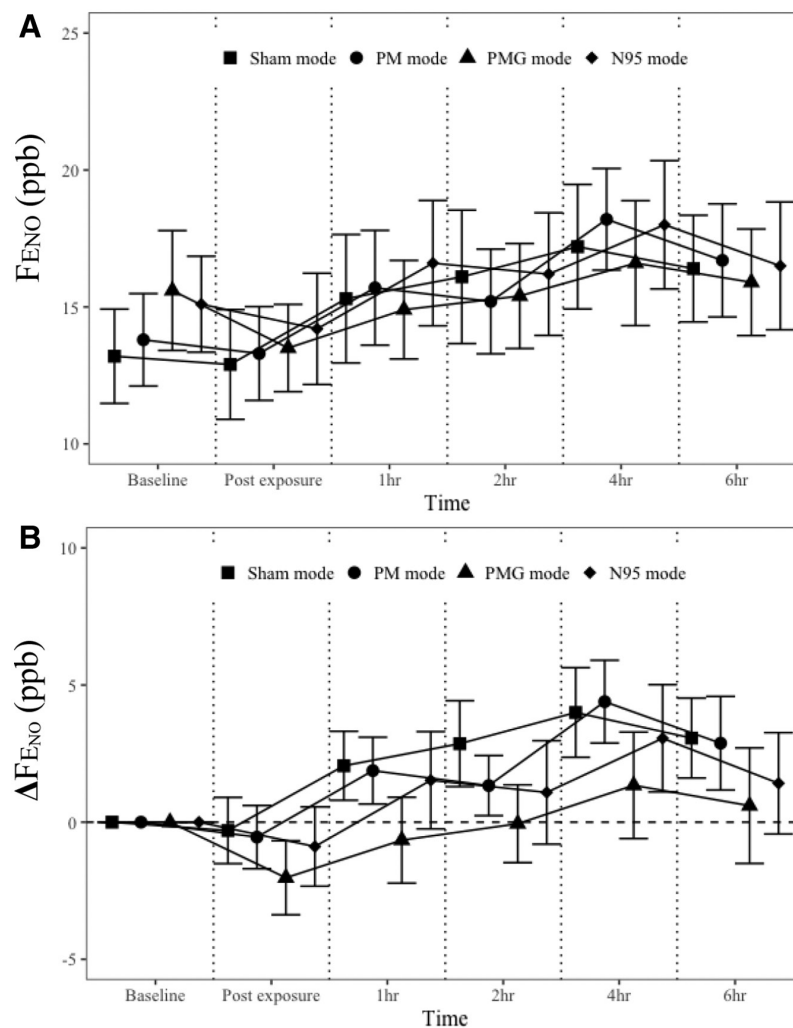
Figure 1. FE_{NO} (A) and differences from baseline FE_{NO} (B) (mean and 95% CI) for each intervention mode and time point (Sham mode: PAPR with no filter in the canister; PM mode: PAPR with filter for only particles; PMG mode: PAPR with filters for both particles and organic gases; N95 mode: wearing an N95 face mask [8210 N95 Respirator, 3M Science, MN]).

Figure 1B is similar to Figure 1A except that differences from baseline are displayed. Distributions of difference from baseline are shown in Table S3; http://links.lww.com/EE/A58. This display shows that in all intervention modes, except the PMG mode, FE_{NO} began to increase 1 hour after the 2-hour exposure, with possibly a peak at 4 hours. Also, in the PMG mode there was no increase from baseline, except possibly at 4 hours following exposure.

Roadside air pollutant concentrations and meteorology

Distributions of roadside pollutant concentrations and meteorology across the intervention modes are presented in Figure 2 and in Table S4; http://links.lww.com/EE/A58. Concentrations of the three air pollutants ($PM_{2.5}$, NO_2, and black carbon) and meteorology were not significantly different across the intervention modes (P-value by ANOVA for $PM_{2.5}$ = 0.11, for NO_2 = 0.12, for black carbon = 0.53, for temperature = 0.38, and for relative humidity = 0.07). Differences in roadside pollutant concentrations and meteorology across intervention mode are accounted for in the mixed model results that follow.

Linear mixed-effects model results

The results of the linear mixed-effect model analyses with only the interventions (and the random effect for participant) in the

model are shown in Figure 3. Only for the PMG intervention mode was the increase in mean FE_{NO} from baseline (mean over all postexposure time points) less than the sham intervention mode (P = 0.002). Also, for the PMG mode, the increase in FE_{NO} from baseline was lower than the sham mode at every time point, although not statistically significant at 6 hours. For the PM and N95 intervention modes, increase in FE_{NO} from baseline was no different from the sham mode, except possibly at 2 hours following exposure (only for N95). The findings were not materially changed after controlling for roadside pollutant concentrations or meteorology (Figure S3).

Figure S4 shows FE_{NO} at baseline for each individual in the morning of each intervention day. While FE_{NO} should be reasonably reproducible within a healthy individual, baseline values in a few individuals were variable. The data on these individuals were retained for the primary analysis, but a sensitivity analysis was performed in which data from individuals who had more than a 10-ppb difference between any two baseline values were excluded from the analysis. As shown in Figure S5, findings were not materially changed following removal of participants with variable baseline FE_{NO}.

Discussion

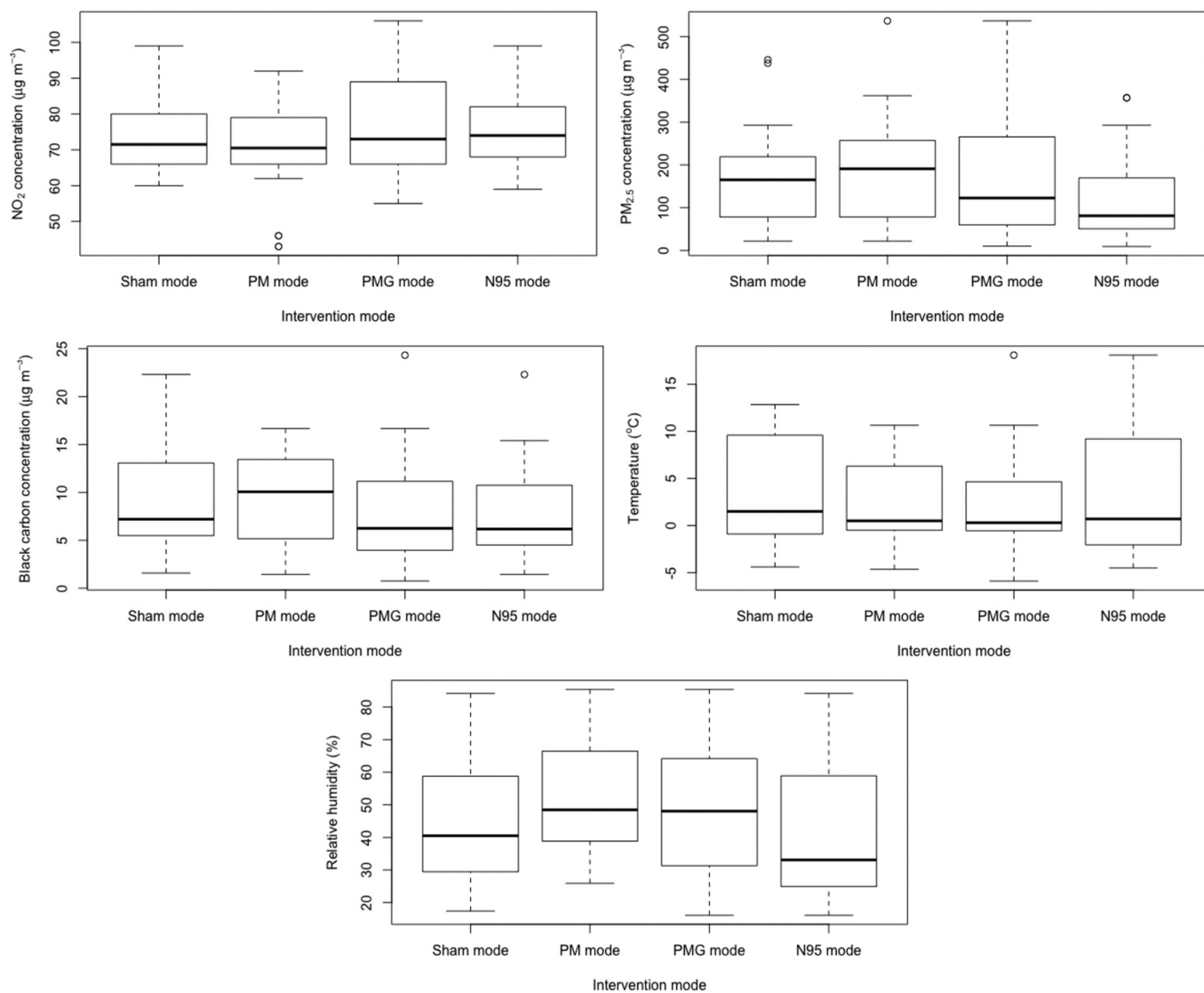The components and sources of air pollution that are most harmful to human health remain largely unknown after many

Figure 2. Roadside pollutant concentrations and meteorology across the four intervention modes.
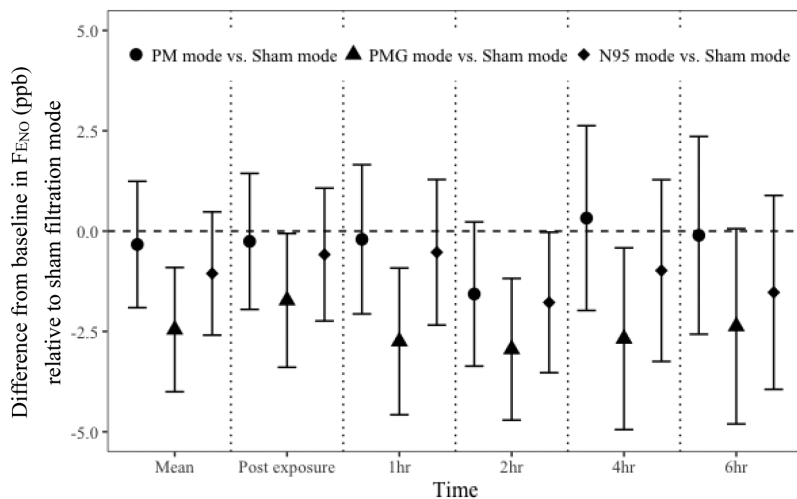

Figure 3. Change (and 95% CIs) in ΔFE_{NO} from baseline by intervention mode relative to the sham mode for the mean of all postexposure FE_{NO} measurements ("Mean") and for each time point.

years of concerted research. TRAP, specifically, has been a source of concern because of the ubiquitous nature of the exposure, and because it contains many pollutants and classes of pollutants that individually are known or strongly suspected to be toxic. While control of TRAP exposure in general would help prevent the health consequences of exposure, an understanding of specific harmful components could allow for more directed control measures. Here, we used respirators that selectively filtered some TRAP components and an experimental study design to attempt to identify broad classes of TRAP components that are harmful. At least as relates to airway inflammation, a central pathway in the mode of action of air pollution, the VOC component of TRAP was identified to be the critical component, rather than the particulate matter component, including road dust, or the inorganic gases such as NO_2.

Most studies on the human health effects of TRAP have used observational designs. Because components of TRAP are highly correlated temporally and spatially, observational designs typically have little ability to implicate specific TRAP components. Arguably, one exception has been the efforts to distinguish the effects of traffic emissions from those of traffic noise.[10] Studies of exposure effects while driving on roadways have similar aims as our study, but most attempt instead to attribute observed effects to measured observed concentrations of specific air pollutant components.[2,11] N95 PM filter masks have been employed in other intervention studies, and while these achieve some control over PM exposure,[12,13] they do not allow blinding to the intervention. PAPRs were used in one other roadway intervention study to control exposure conditions, but because only a PM filter was used, the role of VOCs was not addressed.[7] While admittedly not a practical solution to minimizing exposure to environmental air pollution, the PAPRs here enabled blinding of study participants to the intervention, and the separate stages of filtration that filtered out different components of the TRAP mixture presented an opportunity to distinguish effects of these components.

Another design strength of this study was the use of baseline FE_{NO} to enable the assessment of change from baseline due to traffic exposure across the several interventions, allowing for control of within-person, day-to-day variation in FE_{NO}. FE_{NO} has been considered to be a marker of upregulation of airways inflammation, originating from airway epithelial cells as a result of upregulation of inducible NO synthase that occurs with inflammation.[14] However, there is still uncertainty as to how FE_{NO} should be interpreted, especially in persons without asthma. While the observed increases in FE_{NO} associated with roadway exposure may reflect airways inflammation, the clinical significance of our findings remain open to conjecture.

It was perhaps surprising that the VOC component of the TRAP mixture was critical to the exposure effect on FE_{NO}. Most of the focus and concern in recent years has been on the particulate matter component of the ambient and TRAP mixtures,[7,15–17] but filtering of the PM component did not prevent the increase in FE_{NO}. This finding is somewhat at odds with the findings of the one other intervention study using PAPRs in which PM filtration prevented the on road in-vehicle increase in nitrite and possibly malondialdehyde in exhaled breath condensate (EBC).[7] Possible explanations for these apparently conflicting results include the differences in FE_{NO} and the EBC measures as measures of airway inflammation and oxidative stress, the differences in TRAP exposure within vehicles and at the roadside, and the difference in vehicle mix on the respective roadways in China and New Jersey. Heavy-duty diesel vehicles were prohibited from driving on the roadway during the study period in Tianjin. The study design in effect also allowed us to rule out an effect of inorganic gases such as NO_2 in causing the increase in FE_{NO}. Because the PAPR filtration of PM and VOCs did not filter out inorganic gases, and yet VOC filtration prevented the increase in FE_{NO}, we concluded that the observed effect on FE_{NO} was also not due to the inorganic gases in TRAP. The same logic allowed us to exclude noise as the cause of the increase in TRAP-associated FE_{NO}.

Despite the focus on PM, there has nevertheless been some interest in the non-neoplastic health effects of VOCs. In observational epidemiological studies, exposure to VOCs has been associated with reduced level of lung function in the general population,[18] with cardiorespiratory emergency room visits[19] and additionally with EBC acidity and weakly with FE_{NO} in wheezing children.[20] In a cross-over study of cyclists on high- and low-traffic routes, VOCs, especially benzene, was associated with increased FE_{NO}. Toxicologic studies have also been done using TRAP and other mixtures with effects on the lung being detected for semi-volatile alkanes and volatile aliphatic acids in mice and rats, respectively.[6]

While this study has notable strengths as itemized above, there are some limitations that motivate caution in interpreting and generalizing the findings. The study participants were all young and healthy without respiratory or other illnesses. Apart from the vagaries of interpreting changes in FE_{NO} in those without asthma, as touched on above, the findings are not necessarily applicable to those with asthma or other underlying conditions, or to the elderly. Use of other measures of airway inflammation could help in interpreting and corroborating our findings. Because heavy-duty diesel vehicles were prohibited from using the study roadway, our findings may only be applicable to TRAP from gasoline and natural gas fuel vehicles. Diesel exhaust contains much more PM than gasoline exhaust, but gasoline exhaust has much higher concentrations of VOCs. Filtration of gas phase VOCs was done with filters for both PM and VOCs. It is conceivable, although probably unlikely, that preventing the effect on FE_{NO} as was seen required limiting exposure to gaseous VOCs and PM in concert, and that filtering either alone may not have been sufficient. Future work can overcome this limitation by filtering only gaseous VOCs. Finally, we do not have information on specific VOCs or classes of VOCs that might be more toxic. Attempting to selectively filter out separate chemical groups of VOCs could further hone in on the toxic VOC species of most concern.

Conflicts of interest statement

The authors declare that they have no conflicts of interest with regard to the content of this report.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Health Effects Institute. *Traffic-Related Air Pollution: A Critical Review of the Literature on Emissions, Exposure, and Health Effects*. Boston, MA: Health Effects Institute; 2010.
2. Adar SD, Adamkiewicz G, Gold DR, Schwartz J, Coull BA, Suh H. Ambient and microenvironmental particles and exhaled nitric oxide before and after a group bus trip. *Environ Health Perspect*. 2007;115:507–512.
3. Delfino RJ, Staimer N, Tjoa T, Gillen DL, Schauer JJ, Shafer MM. Airway inflammation and oxidative potential of air pollutant particles in a pediatric asthma panel. *J Expo Sci Environ Epidemiol*. 2013;23:466–473.
4. McCreanor J, Cullinan P, Nieuwenhuijsen MJ, et al. Respiratory effects of exposure to diesel traffic in persons with asthma. *N Engl J Med*. 2007;357:2348–2358.

5. Weichenthal S, Kulka R, Bélisle P, et al. Personal exposure to specific volatile organic compounds and acute changes in lung function and heart rate variability among urban cyclists. *Environ Res*. 2012;118:118–123.

6. Mauderly JL, Seilkop SK. The National Environmental Respiratory Center (NERC) experiment in multi-pollutant air quality health research: III. Components of diesel and gasoline engine exhausts, hardwood smoke and simulated downwind coal emissions driving non-cancer biological responses in rodents. *Inhal Toxicol*. 2014;26:668–690.

7. Laumbach RJ, Kipen HM, Ko S, et al. A controlled trial of acute effects of human exposure to traffic particles on pulmonary oxidative stress and heart rate variability. *Part Fibre Toxicol*. 2014;11:45.

8. American Thoracic Society; European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Critl Care Med*. 2005;171:912–930.

9. Bjermer L, Alving K, Diamant Z, et al. Current evidence and future research needs for FeNO measurement in respiratory diseases. *Respir Med*. 2014;108:830–841.

10. Beelen R, Hoek G, Houthuijs D, et al. The joint association of air pollution and noise from road traffic with cardiovascular mortality in a cohort study. *Occup Environ Med*. 2009;66:243–250.

11. Sarnat JA, Golan R, Greenwald R, et al. Exposure to traffic pollution, acute inflammation and autonomic response in a panel of car commuters. *Environ Res*. 2014;133:66–76.

12. Shi JJ, Lin ZJ, Chen RJ, et al. Cardiovascular benefits of wearing particulate-filtering respirators: a randomized crossover trial. *Environ Health Perspect*. 2017;125:175–180.

13. Langrish JP, Li X, Wang S, et al. Reducing personal exposure to particulate air pollution improves cardiovascular health in patients with coronary heart disease. *Environ Health Perspect*. 2012;120:367–372.

14. Dweik RA, Boggs PB, Erzurum SC, et al; American Thoracic Society Committee on Interpretation of Exhaled Nitric Oxide Levels (FENO) for Clinical Applications. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med*. 2011;184:602–615.

15. Brook RD, Rajagopalan S, Pope CA 3rd, et al; American Heart Association Council on Epidemiology and Prevention, Council on the Kidney in Cardiovascular Disease, and Council on Nutrition, Physical Activity and Metabolism. Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. *Circulation*. 2010;121:2331–2378.

16. Pope CA 3rd, Burnett RT, Thun MJ, et al. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. *JAMA*. 2002;287:1132–1141.

17. Pettit AP, Kipen H, Laumbach R, et al. Disrupted nitric oxide metabolism from type II diabetes and acute exposure to particulate air pollution. *PLoS One*. 2015;10:e0144250.

18. Cakmak S, Dales RE, Liu L, et al. Residential exposure to volatile organic compounds and lung function: results from a population-based cross-sectional survey. *Environ Pollut*. 2014;194:145–151.

19. Ye D, Klein M, Chang HH, et al. Estimating acute cardiorespiratory effects of ambient volatile organic compounds. *Epidemiology*. 2017;28:197–206.

20. Martins PC, Valente J, Papoila AL, et al. Airways changes related to air pollution exposure in wheezing children. *Eur Respir J*. 2012;39:246–253.