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Respiratory Effects of Traffic-Related Air Pollution: A Randomized, Crossover Analysis of Lung Function, Airway Metabolome, and Biomarkers of Airway Injury

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Background: Exposure to traffic-related air pollution (TRAP) has been associated with increased risks of respiratory diseases, but the biological mechanisms are not yet fully elucidated.

Objectives: Our aim was to evaluate the respiratory responses and explore potential biological mechanisms of TRAP exposure in a randomized crossover trial.

Methods: We conducted a randomized crossover trial in 56 healthy adults. Each participant was exposed to high- and low-TRAP exposure sessions by walking in a park and down a road with high traffic volume for 4 h in random order. Respiratory symptoms and lung function, including forced expiratory volume in the first second (FEV_1), forced vital capacity (FVC), the ratio of FEV_1 to FVC, and maximal mid-expiratory flow (MMEF), were measured before and after each exposure session. Markers of 8-isoprostane, tumor necrosis factor-α (TNF-α), and ezrin in exhaled breath condensate (EBC), and surfactant proteins D (SP-D) in serum were also measured. We used linear mixed-effects models to estimate the associations, adjusted for age, sex, body mass index, meteorological condition, and batch (only for biomarkers). Liquid chromatography–mass spectrometry was used to profile the EBC metabolome. Untargeted metabolome-wide association study (MWAS) analysis and pathway enrichment analysis using mummichog were performed to identify critical metabolomic features and pathways associated with TRAP exposure.

Results: Participants had two to three times higher exposure to traffic-related air pollutants except for fine particulate matter while walking along the road compared with in the park. Compared with the low-TRAP exposure at the park, high-TRAP exposure at the road was associated with a higher score of respiratory symptoms [2.615 (95% CI: 0.605, 4.626), $p = 1.2 \times 10^{-2}$] and relatively lower lung function indicators [−0.075 L (95% CI: −0.138, −0.012), $p = 2.1 \times 10^{-2}$] for FEV_1 and −0.190 L/s (95% CI: −0.351, −0.029; $p = 2.4 \times 10^{-2}$) for MMEF]. Exposure to TRAP was significantly associated with changes in some, but not all, biomarkers, particularly with a 0.494-ng/mL (95% CI: 0.297, 0.691; $p = 9.5 \times 10^{-6}$) increase for serum SP-D and a 0.123-ng/mL (95% CI: −0.208, −0.037; $p = 7.2 \times 10^{-3}$) decrease for EBC ezrin. Untargeted MWAS analysis revealed that elevated TRAP exposure was significantly associated with perturbations in 23 and 32 metabolic pathways under positive- and negative-ion modes, respectively. These pathways were most related to inflammatory response, oxidative stress, and energy use metabolism.

Conclusions: This study suggests that TRAP exposure might lead to lung function impairment and respiratory symptoms. Possible underlying mechanisms include lung epithelial injury, inflammation, oxidative stress, and energy metabolism disorders. https://doi.org/10.1289/EHP11139

Introduction

Respiratory disease is a major contributor to the global burden of disease.[1] Ambient air pollution, especially, has been recognized as an important risk factor for respiratory health.[2–5] Traffic emission is the major sour ce of urban air pollution and exposure to traffic-related air pollution (TRAP) has been associated with various respiratory diseases worldwide.[6–8] However, most existing studies on air pollution and respiratory morbidity and mortality have been observational and thus had limited ability to establish a causal relationship between exposure and outcome.[9,10] Many studies have been conducted under controlled-exposure experimental settings (e.g., by using exposure chambers to create TRAP exposure contrast among the participants).[11–14] However, most of these studies considered only one or two pollutants. Meanwhile, in reality, TRAP is a mixture of air pollutants and the main components include nitrogen oxides (NO_x), carbon monoxide (CO), fine and ultrafine particulate matter [PM ≤2.5 μm in aerodynamic diameter ($PM_{2.5}$) and UFP], and black carbon (BC).[15–17] Two studies in London, UK, have examined respiratory and cardiovascular responses to TRAP by comparing participants' health measures after walking down a busy road and in a traffic-free area.[18,19] However, their findings may not be generalizable in other countries or regions owing to the differences in vehicle fuel composition, levels of exposure, and population characteristics.

The underlying mechanisms of respiratory effects caused by TRAP have not been fully clarified. The metabolomics technique has emerged as a powerful tool to detect molecular changes following perturbations, such as environmental exposures, comprehensively. However, few studies have fully applied this technique to examine the molecular changes with TRAP exposure.[20–22] In addition, only one study has considered profiling metabolites in exhaled breath condensate (EBC) samples to explore changes in the respiratory system.[23] Therefore, more evidence is warranted.

To address these knowledge gaps, we conducted a randomized, crossover study in Shanghai, China, to examine the associations of the respiratory effects associated with TRAP exposure

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and to explore the underlying mechanisms by analyzing the metabolome in EBC.

Methods

Study Design and Participants

We initially recruited 69 healthy, nonsmoking college students (>18 years of age) from the medical campus of Fudan University in Shanghai, China. Students who had no history of self-reported allergy or chronic cardiopulmonary diseases, who had lived on campus for at least 1 y, who had no secondhand smoking exposure in their main indoor environments (i.e., dormitory and office), and who had not used any medication or dietary supplements in the recent 2 months were eligible to enroll. We measured their height and weight for calculating body mass index (BMI) and collected their demographic and medical information through a questionnaire that included age, sex, smoking, history of disease (e.g., cardiorespiratory and allergic diseases, major surgeries), and medication and dietary supplement use in the recent 2 months. The questionnaire survey can be found in the Supplemental Materials, "Basic Information Questionnaire." In addition, we conducted pulmonary function tests for all participants at enrollment to screen for those with abnormal lung function or inability in test performing. The pulmonary function test was conducted according to the recommendation of the American Thoracic Society/European Respiratory Society (ATS/ERS)[24] using a portable spirometer (Jaeger MasterscreenV5.01, CareFusion). Six participants were then excluded because their lung function test results were below the normal range [i.e., the forced expiratory volume in the first second (FEV_1) to forced vital capacity (FVC) ratio was <0.65 or the predicted FEV_1 was <75%] and another 7 participants declined to participate. Eventually, 56 participants were included in the trial.

Each participant was required to finish a high-and a low-TRAP exposure session in random orders intermitted by no less than a 14-d washout period between October and December 2019 (Figure S1). For the high-TRAP exposure session, participants were assigned to walk on the sidewalk along North Caoxi Road, which is located in urban Shanghai and has ~77,400 vehicles on the road per day. For the low-exposure session, the participants were led to walk in Century Park following a preassigned walking route 0.5–1.5 km away from traffic roadways.[25] For feasibility, participants were divided randomly into 14 groups (1–3 persons per group) and each group was arranged to complete the exposure sessions on different days. In each session, participants were asked to walk at a steady pace for 15 min followed by a 30-min rest for 4 h (from 1300 to 1700 hours). To minimize noise exposure, we provided earplugs during each walking session.

To minimize the differential exposure to TRAP prior to each session, all the participants were asked to stay on campus for at least 3 d before each session. To reduce the influence of other potential confounders, all the participants were asked to provide information that might cause short-term systemic inflammation, including disease conditions, use of alcohol, medication and dietary supplements, and passive smoking, in the past 3 d prior to each exposure session by questionnaire (Supplemental Materials, "Information before each exposure session"). Only those who had not taken medication, dietary supplements, consumed alcohol, been exposed to secondhand smoke, or had symptoms of illness within the past 3 d were allowed to participate in the upcoming exposure session. To minimize possible impacts from the diet, we also provided standardized meals and water to all participants on the day of the exposure session.

The study protocol was registered at ClinicalTrials.gov (NCT04153539). The institutional review board of the School of Public Health, Fudan University, approved the study protocol (IRB#2019-07-0768). All participants provided written informed consent at enrollment.

Exposure Measures

During each exposure session, a trained staff member carried a backpack equipped with portable devices to measure real-time air pollution exposures. $PM_{2.5}$ mass concentration was measured by MicroPEM (RTI International), which is a portable one-stage impactor equipped with an onboard micro-nephelometer. UFP number concentration was measured by NanoTracer (XP; Oxility), which records the number concentrations of particles with a size between 20 nm and 120 nm every 10 s using the diffusion charging method. BC concentration was measured using MicroAeth (AE51; AethLabs), which detects the transmission of light at the 880-nm wavelength through the active area of a filter (referred to as the sensing channel) of aerosol collection. Nitrogen dioxide (NO_2) and CO were measured by dynamic baseline electrochemical sensors based on electrochemical sensing and pair differential filter technology, respectively (Sapiens PEK Lite A1; Sapiens Environmental Technology Co. Ltd.).[26] The meteorological variables, including ambient temperature and relative humidity, were also collected using a HOBO data logger (Onset Computer Corporation). The 4-h average of pollutant concentrations for each exposure session was calculated for the statistical analysis.

All devices were tested for comparability and calibrated for data quality control. We conducted side-by-side tests on devices for each air pollutant measurement under laboratory conditions to evaluate the reproducibility within units in a scenario without any major indoor pollutant sources. For each type of device, all the units were placed on a table at the center of the lab (6 m × 5 m × 3 m) and set to run for a continuous 24 h with a fixed data acquirement interval (i.e., 5 min for NanoTracer, 1 min for MicroPEM, 5 min for MicroAeth, and 1 min for Sapiens PEK Lite). We kept all the windows and doors closed to minimize potential influence from airflow disturbance. We conducted tests for comparability of devices three times in total, that is, before the trial (September 2019), at the middle of the trial (November 2019), and at the end of the trial (December 2019). To reduce the exposure misclassification, two units showing the best interconsistency in the comparability tests were used in the trial (Figures S2–S4).

Health Measurements

Respiratory symptoms. We used a questionnaire adapted from the Swedish Performance Evaluation System (SPES)[27] to measure the degree of respiratory symptoms. This questionnaire requires participants to use a scale of 0–5 to rate their self-perceived severity of 14 respiratory tract symptoms, including irritation of the nose, itchy nose, dry nose, burning nose, stuffy nose, running nose, itchy throat, irritation throat, swelling throat, burning throat, urge to cough, pressure on the chest, oppression to breath, and expectoration. A higher score indicates greater symptom severity, with a score of 0 referring to no symptoms and a score of 5 referring to a severe symptom. The total respiratory symptom score was then calculated by summing up the scores across the 14 symptoms. The questionnaires were completed half an hour before and immediately after each exposure session.

Lung function test. Lung function was measured before and after each exposure session in a lab located on campus using a portable spirometer (Jaeger MasterscreenV5.01; CareFusion) under the instruction of the same trained operator. The lung function test was conducted according to the recommendation of the ATS/ERS.[24] Specifically, participants were instructed to make three attempts intermitted by at least 3 min and a qualified measure

should have at least two acceptable and reproducible test results from all attempts. The measures of lung function included in this study were FEV_1, an indicator of airway obstruction; FVC, an indicator of airway restriction; and maximal mid-expiratory flow (MMEF), an indicator of small airway function. We also calculated the ratio of FEV_1 and FVC (FEV_1/FVC) to examine airway obstruction.

Sample collection and analysis. EBC and peripheral blood samples were collected before and after each exposure session within 1 h in a lab after returning to the campus. All participants were required to refrain from eating or drinking for at least 2 h before the testing and specimen collection. The EBC was collected by trained staff using an exhaled breath condensate collection system (Erich Jaeger GmbH) following the ATS/ERS recommended guidelines.[28] Participants were asked to rinse their mouths before sample collection to avoid saliva contamination. During the sample collection, participants were instructed to breathe into the collection system using tidal breathing to ensure that the volume of EBC was reproducible within individuals. The collection system runs at -5°C to condense water vapor in the exhaled breath. All EBC samples were stored at -80°C within 2 h after collection. Blood samples (5 mL) were collected by a professional medical staff member (a clinical nurse) using the serum separator tube (BD Vacutainer SST II advance) and were processed for serum extraction immediately after collection and then stored at -80°C until analysis.

Biomarkers in the EBC and blood samples collected before and after each exposure session were quantified using an enzyme linked immunosorbent assay (ELISA) according to the operating manual provided by the manufacturers. Specifically, serum surfactant proteins D (SP-D) (DSFPD0; R&D Systems) and EBC ezrin (SEB297Hu; Cloud Clone Corp.) are indicators of lung epithelial injury, whereas EBC 8-isoprostane (516351; Cayman Chemical) and tumor necrosis factor-α (TNF-α) (HSTA00E; R&D Systems) are markers of airway oxidative stress and inflammation, respectively. All samples collected from the same participant were processed in the same batch. All samples were analyzed twice according to the protocol of the manufacturer and a blank well was set in each batch and all samples (including the blank and standard controls). The measured value of each well was subtracted by the average value of the blank controls. Then means of two duplicated samples were used as the final measurement results in the statistical analysis. The coefficients of variation (CVs, %) for SP-D, 8-isoprostane, ezrin, and TNF-α detection were all <10%. The limits of detection (LODs) for SP-D, 8-isoprostane, ezrin, and TNF-α detection were 0.123 ng/mL, 0.8 pg/mL, 0.312 ng/mL, and 0.049 pg/mL, respectively. Half of the LOD was used to replace those measurements below the LOD. Eventually, only measurements of two samples on ezrin and TNF-α were below the LOD and were replaced.

EBC samples collected after each exposure session were used for metabolomic analysis in ultra-high performance liquid chromatography–mass spectrometry (UHPLC-MS/MS) (Vanquish; Thermo Fisher Scientific; and Orbitrap MS; Thermo). The detailed description on LC-MS/MS analysis can be found elsewhere.[29] In brief, LC-MS/MS analyses were performed using an UHPLC system (Vanquish; Thermo Fisher Scientific) with a UPLC BEH Amide column (2.1 mm × 100 mm, 1.7 μm) coupled to a Q Exactive HF-X (QE HFX) mass spectrometer (Orbitrap MS; Thermo). The QE HFX mass spectrometer was used owing to its ability to acquire MS/MS spectra in information-dependent acquisition mode in the control of the acquisition software (Xcalibur; Thermo). To validate the quality of measurements, quality control samples were generated by mixing aliquots of all samples with equal volume. The same pretreatment method was applied for quality control samples and samples to be tested. Pooled quality control samples were inserted in every several samples to assess the repeatability of the instrument. ProteoWizard was adopted to convert the raw data into the mzXML format and processed with a XCMS-based program for peak extraction. We excluded the features with missing values >50%. Half of the minimum value was used to replace each of the missing values. All data were Pareto-scaled before statistical analysis.

Statistical Analysis

We first calculated the differences in respiratory system symptom scores, lung function measures, and concentrations of biomarkers (i.e., EBC ezrin, EBC 8-isoprostane, EBC TNF-α, and serum SP-D) before and after each exposure session (i.e., adjusting for the baseline). Then we applied linear mixed-effect (LME) models to estimate the associations of TRAP exposure with changes in each of the aforementioned health measures. Specifically, TRAP exposure was first fitted as a binary indicator of exposure session (0 for low-exposure session and 1 for high-exposure session) in the model to compare the difference of changes in these measures under the two exposure scenarios. In addition, we fitted separate models with continuous variables of air pollutants (UFP, BC, $PM_{2.5}$, NO_2, or CO) at each exposure session (i.e., averages of pollutants concentrations over the 4-h exposure session) as the exposure of interest. For all models, we adjusted for demographic characteristics [i.e., age (continuous variable), sex (binary variable), and BMI (continuous variable)] to control for the potential heterogeneity between individuals, and meteorological conditions [i.e., temperature (continuous variable) and relative humidity (continuous variable)] to control for the potential heterogeneity between days. For biomarkers, we additionally adjusted for batch number in the fixed effect terms. A random intercept for each participant was also added into the model to account for within-participant correlations.[19,30] Last, we fitted nonlinear models for each pollutant using a natural spline function, adjusting for the same set of covariates. The likelihood ratio test was used to examine for possible nonlinear exposure–response relationships, and the results suggested no statistically significant nonlinear exposure–response relationship (Table S1). All analyses were implemented in R software (version 3.4.4; R Development Core Team) using the package lme4. Effect estimates for the biomarkers were expressed as mean absolute changes with 95% confidence intervals (CIs) associated with TRAP exposure and per interquartile range (IQR) increase of air pollutants.

The untargeted metabolome-wide association study (MWAS) was conducted for EBC metabolomics. We obtained the mass-to-charge ratio (*m/z*), retention time, and ion intensity for each detected metabolic feature. Features that were detected in <50% of the samples were excluded. Ion intensities of the remaining features were log-transformed for normalization and were then added into separate LME models as dependent variables to examine their associations with TRAP exposure (binary) and with each pollutant (continuous),[31] adjusting for the same set of covariates. The Benjamini–Hochberg false discovery rate (FDR) was calculated to control for multiple comparisons, and an FDR of <0.05 was considered statistically significant.

To identify the underlying biological pathways related to TRAP exposure, we used the mummichog pathway analysis (version 1.0.10; Python) for pathway enrichment analysis. Different from the traditional targeted metabolomic analysis strategy, the mummichog approach uses algorithms that leverage known metabolic pathways and networks to predict the function of each metabolite without identifying the metabolites *a priori*.[32] This method has been commonly used in previous studies for predicting network activity from untargeted metabolomic analyses.[31,33–35] We

Table 1. Demographic characteristics of participants ($n = 56$) in a randomized controlled trial of exposure to traffic-related air pollution in Shanghai, China, 2019.

Variables	Mean ± SD or n (%)
Sex	
Male	25 (44.6)
Female	31 (55.4)
Age (y)	23.5 ± 2.4
BMI (kg/m^2)	21.8 ± 2.9

Note: BMI, body mass index; SD, standard deviation.

applied the mummichog approach to screen for the features that differ significantly between high-and low-TRAP exposure sessions or that were associated with air pollutants, under positive- and negative-ion modes, respectively. We applied Fisher's exact test (FET) as an enrichment test of metabolic features on pathways, adjusting for type I error based on a method by Berriz et al.[36] An adjusted $p < 0.05$ from FET was considered statistically significant. Metabolite features associated with enriched metabolic pathways were then annotated in the human metabolome database (HMDB), the METLIN database, and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.[37]

Results

Descriptive Statistics

As shown in Table 1, the mean ± standard deviation (SD) of the age and BMI of participants were 23.5 ± 2.4 y and 21.8 ± 2.9 kg/m^2, respectively. Among the included 56 participants, 25 were males. No participant reported alcohol, medication and dietary supplement use, or exposure to smoking during the study period. During the road exposure session (i.e., high-TRAP exposure), the concentrations of UFP, BC, NO_2, and CO were approximately two to three times higher than in the park exposure session (i.e., low-TRAP exposure), whereas $PM_{2.5}$ concentration showed a relatively smaller difference, with only a 40% higher exposure in the road session compared with the park exposure session. Temperature and relative humidity were comparable in both sessions (Table 2). Spearman rank-correlation coefficients (r) between the five air pollutants ranged from 0.25 to 0.87 (Table S2).

Respiratory Symptoms in Relation to TRAP Exposure

Overall, total respiratory symptom scores before the road and park exposure sessions were similar (mean ± SD = 1.70 ± 2.28 for the road exposure session and 1.89 ± 3.00 for the park exposure session, respectively; Table S3). At the end of each session, the mean total symptom score increased by 3.21 for the road exposure session; the scores for dry nose, itchy throat, and urge to cough had the largest increases. However, no notable increase in total score was found for the park exposure session. Table 3 shows changes in the total score of respiratory symptoms related to TRAP and individual pollutants. Compared with changes in respiratory symptom scores before and after the park exposure session, we observed an increase of 2.615 (95% CI: 0.605, 4.626; $p = 1.2 \times 10^{-2}$) in the changes in symptoms scores for the road exposure session. In addition, we found larger changes of symptom scores were associated with exposure to UFP (IQR: 16,934 particles/cm^3; score difference per IQR increase: 1.793; 95% CI: 0.113, 3.474; $p = 3.9 \times 10^{-2}$), BC (IQR: 3 μg/m^3; score per IQR increase difference: 2.917; 95% CI: 0.873, 4.960; $p = 6.2 \times 10^{-3}$), and NO_2 (IQR: 31 ppb; score difference per IQR increase: 3.360; 95% CI: 1.252, 5.467; $p = 2.3 \times 10^{-3}$) than with exposure to CO (IQR: 632 ppb; score difference per IQR increase: 1.413; 95% CI: −0.611, 3.436; $p = 1.7 \times 10^{-1}$) and $PM_{2.5}$ (IQR: 8 μg/m^3; score difference per IQR increase: 0.503; 95% CI: −0.294, 1.301; $p = 2.2 \times 10^{-1}$), respectively.

TRAP Exposure and Lung Function

We found no notable differences in lung function measures before the road and park exposure sessions (Table 4). Compared with those measured before the exposure sessions, we observed lower FVC, FEV_1, and MMEF in our participants after the road exposure session, whereas no drastic changes were found between all lung function measures before and after the park exposure session. After adjusting for all covariates in LME models, we found TRAP exposure was associated with lower FEV_1 and MMEF (Table 3). For example, compared with lung function changes in the park exposure session, FEV_1 and MMEF decreased by 0.075 L (95% CI: −0.138, −0.012; $p = 2.1 \times 10^{-2}$) and 0.190 L/s (95% CI: −0.351, −0.029; $p = 2.4 \times 10^{-2}$) after the road exposure session.

In addition, among specific air pollutants, we found that NO_2 was significantly associated with a decline in three of the four measures of lung function. For example, an IQR (31 ppb) increase of NO_2 was associated with a 0.035-L (95% CI: −0.107, 0.036; $p = 3.4 \times 10^{-1}$), 0.071-L (95% CI: −0.135, −0.007; $p = 3.2 \times 10^{-2}$), 0.878 (95% CI: −1.630, −0.125; $p = 2.6 \times 10^{-2}$), and 0.203-L/s (95% CI: −0.369, −0.036; $p = 2.0 \times 10^{-2}$) decrease in FVC, FEV_1, FEV_1/FVC, and MMEF, respectively. We also found most effect estimates of other air pollutants on lung function measures were negative; however, their associations were suggestive or null. For example, an IQR (16,934 particles/cm^3) increase of UFP was associated with a

Table 2. Exposure conditions in high (road)- and low (park)-traffic-related air pollution (TRAP) exposure sessions for $n = 56$ adults participating in a randomized crossover trial in China.

Variable	Group	Mean	SD	Min	P25	P50	P75	Max
UFP (particles/cm^3)	Road	33,467	6,678	24,555	28,651	31,608	35,564	49,885
	Park	14,996	4,549	8,123	11,695	14,603	17,488	24,460
BC (μg/m^3)	Road	4	1	3	4	4	5	9
	Park	2	0	1	1	1	2	3
NO_2 (ppb)	Road	44	9	23	39	45	49	59
	Park	14	3	8	11	14	15	21
CO (ppb)	Road	948	196	676	804	928	1,053	1,521
	Park	333	156	41	234	296	450	654
$PM_{2.5}$ (μg/m^3)	Road	27	19	11	17	22	26	98
	Park	19	9	6	13	18	20	56
Temperature (°C)	Road	22	4	12	20	22	24	26
	Park	22	3	13	21	22	24	25
Relative humidity (%)	Road	42	10	25	38	41	46	69
	Park	49	13	31	40	46	54	79

Note: BC, black carbon; CO, carbon monoxide; IQR, interquartile range; max, maximum; min, minimum; NO_2, nitrogen dioxide; P25, 25th percentile; P50, 50th percentile; P75, 75th percentile; $PM_{2.5}$, fine particulate matter; SD, standard deviation; UFP, ultrafine particles.

Table 3. Baseline-adjusted mean changes (95% confidence intervals) in total respiratory symptom score, lung function measures, and biomarkers associated with exposure sessions (road vs. park, $n = 56$ adults) and with per IQR increase of air pollutants in a randomized crossover trial in China.

	TRAP [park(0)/road(1)]		UFP (IQR: 16,934 particles/cm^3)		BC (IQR: 3 μg/m^3)		NO_2 (IQR: 31 ppb)		CO (IQR: 632 ppb)		$PM_{2.5}$ (IQR: 8 μg/m^3)	
	β (95% CI)	*p*-Value	β (95% CI)	*p*-Value	β (95% CI)	*p*-Value	β (95% CI)	*p*-Value	β (95% CI)	*p*-Value	β (95% CI)	*p*-Value
Total symptoms score	2.615 (0.605, 4.626)	1.2×10^{-2}	1.793 (0.113, 3.474)	3.9×10^{-2}	2.917 (0.873, 4.960)	6.2×10^{-3}	3.360 (1.252, 5.467)	2.3×10^{-3}	1.413 (−0.611, 3.436)	1.7×10^{-1}	0.503 (−0.294, 1.301)	2.2×10^{-1}
Lung function												
FVC (L)	−0.060 (−0.130, 0.009)	9.4×10^{-2}	−0.022 (−0.081, 0.037)	4.6×10^{-1}	−0.017 (−0.088, 0.054)	6.4×10^{-1}	−0.035 (−0.107, 0.036)	3.4×10^{-1}	−0.008 (−0.078, 0.062)	8.2×10^{-1}	0.018 (−0.005, 0.041)	1.2×10^{-1}
FEV_1 (L)	−0.075 (−0.138, −0.012)	2.1×10^{-2}	−0.036 (−0.090, 0.017)	1.8×10^{-1}	−0.041 (−0.106, 0.023)	2.1×10^{-1}	−0.071 (−0.135, −0.007)	3.2×10^{-2}	−0.021 (−0.085, 0.042)	5.1×10^{-1}	0.017 (−0.009, 0.044)	2.0×10^{-1}
FEV_1/FVC	−0.571 (−1.290, 0.148)	1.3×10^{-1}	−0.396 (−1.022, 0.231)	2.2×10^{-1}	−0.636 (−1.389, 0.116)	1.0×10^{-1}	−0.878 (−1.630, −0.125)	2.6×10^{-2}	−0.515 (−1.252, 0.222)	1.8×10^{-1}	−0.225 (−0.567, 0.117)	2.0×10^{-1}
MMEF (L/s)	−0.190 (−0.351, −0.029)	2.4×10^{-2}	−0.083 (−0.222, 0.057)	2.5×10^{-1}	−0.153 (−0.319, 0.012)	7.4×10^{-2}	−0.203 (−0.369, −0.036)	2.0×10^{-2}	−0.111 (−0.274, 0.053)	1.9×10^{-1}	−0.005 (−0.076, 0.066)	8.9×10^{-1}
Biomarkers												
Serum SP-D (ng/mL)	0.494 (0.297, 0.691)	9.5×10^{-6}	0.311 (0.126, 0.496)	1.8×10^{-3}	0.476 (0.273, 0.680)	2.9×10^{-5}	0.498 (0.280, 0.715)	4.2×10^{-5}	0.381 (0.164, 0.597)	1.1×10^{-3}	0.070 (−0.031, 0.171)	1.8×10^{-1}
EBC ezrin (ng/mL)	−0.123 (−0.208, −0.037)	7.2×10^{-3}	−0.111 (−0.181, −0.042)	2.7×10^{-3}	−0.135 (−0.218, −0.052)	2.4×10^{-3}	−0.131 (−0.217, −0.046)	1.8×10^{-3}	−0.148 (−0.236, −0.060)	3.8×10^{-3}	−0.028 (−0.064, 0.008)	1.3×10^{-1}
EBC 8-isoprostane (pg/mL)	0.190 (−0.004, 0.385)	6.1×10^{-2}	0.171 (0.008, 0.334)	4.5×10^{-2}	0.191 (−0.001, 0.383)	5.6×10^{-2}	0.205 (0.005, 0.405)	4.9×10^{-2}	0.219 (0.030, 0.409)	2.7×10^{-2}	0.070 (−0.010, 0.151)	9.2×10^{-2}
EBC TNF-α (pg/mL)	0.007 (−0.008, 0.023)	3.6×10^{-1}	0.004 (−0.009, 0.017)	5.1×10^{-1}	0.003 (−0.012, 0.018)	6.9×10^{-1}	0.009 (−0.006, 0.024)	1.8×10^{-1}	0.011 (−0.005, 0.027)	2.6×10^{-1}	0.003 (−0.003, 0.010)	2.9×10^{-1}

Note: All the linear mixed-effect models were adjusted for age, sex, body mass index, batch number (only for biomarkers), and a random intercept for each participant and the previous 4-h moving average of temperature and relative humidity. IQR were calculated based on data measured at both high-and low-exposure sessions. BC, black carbon; CI, confidence interval; CO, carbon monoxide; EBC, exhaled breath condensate; FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity; IQR, interquartile range; MMEF, maximal mid-expiratory flow; NO_2, nitrogen dioxide; $PM_{2.5}$, fine particulate matter; SP-D, surfactant proteins D; TNF-α, tumor necrosis factor-α; TRAP, traffic-related air pollution; UFP, ultrafine particles.

Table 4. Lung function measures and airway biomarkers (mean ± SD) before and after each exposure session (road and park) for $n = 56$ adults participating in a randomized crossover trial in Shanghai, China.

	Road		Park	
	Before	After	Before	After
Lung function				
FVC (L)	4.00 ± 0.91	3.92 ± 0.87	3.94 ± 0.89	3.91 ± 0.92
FEV_1 (L)	3.41 ± 0.68	3.34 ± 0.65	3.32 ± 0.62	3.32 ± 0.67
FEV_1/FVC	86.05 ± 6.90	86.28 ± 6.60	85.40 ± 7.41	86.02 ± 6.76
MMEF (L/s)	3.62 ± 1.03	3.54 ± 0.86	3.39 ± 0.89	3.48 ± 0.87
Biomarkers				
Serum SP-D (ng/mL)	6.52 ± 4.26	6.53 ± 4.59	6.96 ± 5.15	6.51 ± 5.26
EBC ezrin (ng/mL)	0.38 ± 0.28	0.38 ± 0.29	0.33 ± 0.23	0.42 ± 0.24
EBC 8-isoprostane (pg/mL)	1.52 ± 0.58	1.59 ± 0.64	1.66 ± 0.62	1.55 ± 0.59
EBC TNF-α (pg/mL)	0.05 ± 0.03	0.06 ± 0.03	0.06 ± 0.04	0.06 ± 0.03

Note: EBC, exhaled breath condensate; FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity; MMEF, maximal mid-expiratory flow; SD, standard deviation; SP-D, surfactant proteins D; TNF-α, tumor necrosis factor-α.

0.022-L (95% CI: −0.081, 0.037; $p = 4.6\times10^{-1}$), 0.036-L (95% CI: −0.090, 0.017; $p = 1.8\times10^{-1}$), 0.396 (95% CI: −1.022, 0.231; $p = 2.2\times10^{-1}$), and 0.083-L/s (95% CI: −0.222, 0.057; $p = 2.5\times10^{-1}$) decreases in FVC, FEV_1, FEV_1/FVC, and MMEF, respectively.

TRAP Exposure and Airway Biomarkers

Table 3 shows the baseline-adjusted mean changes of serum SP-D, EBC ezrin, 8-isoprostane, and TNF-α associated with exposure sessions and specific air pollutants. Compared with the park exposure session, we found participants had lower EBC ezrin (mean difference = −0.123 ng/mL; 95% CI: −0.208, −0.037; $p = 7.2\times10^{-3}$) and higher EBC 8-isoprostane (mean difference = 0.190 pg/mL; 95% CI: −0.004, 0.385; $p = 6.1\times10^{-2}$), and serum SP-D (mean difference = 0.494 ng/mL; 95% CI: 0.297, 0.691; $p = 9.5\times10^{-6}$), respectively, after the road exposure session. Consistently, UFP, BC, NO_2, and CO exposures were all associated with higher serum SP-D ($p = 1.8\times10^{-3}$, 2.9×10^{-5}, 4.2×10^{-5}, and 1.1×10^{-3}, respectively) and EBC 8-isoprostane ($p = 4.5\times10^{-2}$, 5.6×10^{-2}, 4.9×10^{-2}, and 2.7×10^{-2}, respectively) and lower EBC ezrin ($p = 2.7\times10^{-3}$, 2.4×10^{-3}, 1.8×10^{-3}, and 3.8×10^{-3}, respectively). However, no significant changes were found in TNF-α related to the exposure sessions and specific air pollutants. In addition, the associations between biomarkers and $PM_{2.5}$ exposure showed null.

TRAP Exposure and Airway Metabolome

In total, we detected 2,305 and 2,098 metabolite features from positive- and negative-ion modes, respectively. Among them, 936 (positive mode) and 945 (negative mode) metabolic features, respectively, were identified as differential metabolic features between the road and park exposure sessions. The mummichog pathway analysis further identified perturbations in 23 and 32 metabolic pathways based on these features under the positive- and negative-ion modes, respectively (Figure 1; Table S4). These enriched metabolic pathways were closely related to the systemic inflammatory response (e.g., arginine and proline metabolism, and leukotriene metabolism), oxidative stress (e.g., linoleate metabolism, purine metabolism, methionine and cysteine metabolism, and vitamin E metabolism), and energy metabolism [e.g., tricarboxylic acid (TCA) cycle].

Among these enriched metabolic pathways, 156 and 129 unique metabolic features were associated with TRAP air pollutants in the positive- and negative-ionization modes, respectively.

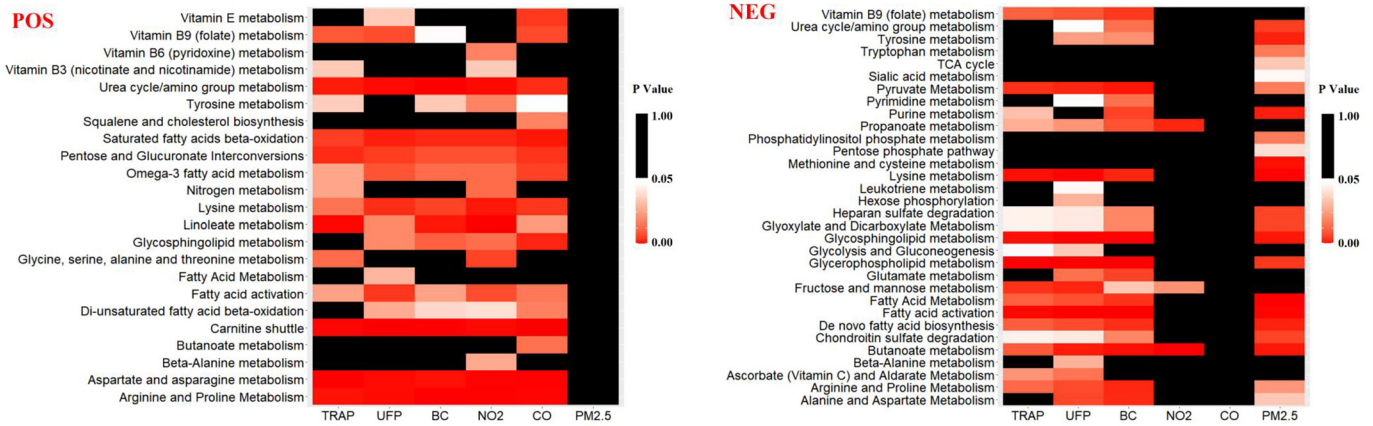

Figure 1. The identified metabolic pathways associated with TRAP and individual air pollutants in positive-ionization mode (POS) and negative-ionization mode (NEG) based on the untargeted metabolome-wide association study (MWAS) conducted for exhaled breath condensate (EBC) metabolomics in a randomized crossover trial in China ($n = 56$ adults). TRAP exposure was fitted as a binary indicator of exposure (low/high); individual pollutants were modeled as continuous variables. The linear mixed-effect models and the mummichog pathway analysis (version 1.0.10; Python) were applied for pathway enrichment analysis. Fisher's exact test (FET) as an enrichment test of metabolic features on pathways was applied, and an adjusted $p < 0.05$ from FET was considered statistically significant. *p*-Values are shown in Table S4. Note: BC, black carbon; CO, carbon monoxide; NO_2, nitrogen dioxide; $PM_{2.5}$, fine particulate matter; TCA, tricarboxylic acid; TRAP, traffic-related air pollution; UFP, ultrafine particles.

Based on the enriched metabolic pathways, we further confirmed 27 of them were associated with TRAP (Table 5). Most of these features were endogenous metabolites related to inflammatory, oxidative stress, and energy metabolism. For example, we found metabolites related to inflammation and oxidative stress, including leukotriene B_4, 13-hydroxyoctadecadienoic acid, hypoxanthine, uric acid, and L-arginine, increased after the road exposure session and with individual pollutants. Meanwhile, metabolites that were suggested to have anti-inflammation and anti-oxidative stress properties (e.g., γ-linolenic acid) were found to be reduced after the road exposure session. Moreover, metabolites related to energy metabolism (e.g., fumarate, succinate, D-glucose, lactate, pyruvate) were also found to increase after the road exposure session and with higher UFP and BC concentrations.

Discussion

In this study, we conducted a randomized crossover trial to explore respiratory responses related to TRAP exposure among healthy adults. After comparing changes before and after the road and park exposure sessions, we found TRAP exposure was associated with higher respiratory symptom scores and lower lung function. Further, biomarkers related to lung epithelial injury, airway inflammation, and oxidative stress increased after exposure to TRAP. Metabolomics analysis in EBC samples identified metabolic signals and pathways closely related to inflammation, oxidative stress, and energy metabolism (Figure 2). We also found UFP, BC, NO_2, and CO, but not $PM_{2.5}$, were significantly associated with changes in respiratory health markers, and NO_2 had stronger associations with changes in respiratory symptom scores and lung function measures.

Respiratory Symptoms and Lung Function Changes Related to TRAP Exposure

Previous epidemiological studies have found that exposure to TRAP was associated with respiratory symptoms.[38–40] However, those studies were mostly observational and focused on vulnerable populations (e.g., children, asthmatics) in Europe. Therefore, it may not be feasible to directly compare their results with our findings. A previous randomized, crossover trial in the UK assessed the effects on respiratory and cardiovascular responses of TRAP exposure among healthy elderly and elderly with chronic lung or heart disease, and it found short-term exposure to TRAP was associated with symptoms in the respiratory tract (e.g., cough, sputum, wheeze) and lower lung function.[18] Consistent with our results, that UK study also illustrated that the benefits of walking exercise on lung function might be offset by TRAP exposure among adults.[18]

Similarly, we found a significant decrease in lung function following acute TRAP exposure. These lung function changes indicate airway obstruction (manifested by a significant decrease in FEV_1 and no significant change in FVC) and small airway changes (manifested by a significant decrease in MMEF). MMEF has been considered a sensitive physiological marker of small airway function decline.[41] Therefore, the observed declined MMEF associated with TRAP exposure suggests TRAP exposure might be associated with early pathophysiological impairment of small airways among healthy adults.[42]

Potential Biological Mechanisms of TRAP-Related Respiratory Effect

We found significant changes of serum SP-D and EBC ezrin associated with TRAP exposure. Both serum SP-D and EBC ezrin are biomarkers of airway epithelial damage. SP-D regulate airway surface tension.[43,44] Previous studies in humans have suggested SP-D were inversely associated with lung function.[45,46] Ezrin is related to the integrity of the airway epithelial barrier by maintaining the normal morphology and intercellular adhesion of epithelial cells.[47,48] We hypothesize that the increase of serum SP-D and decrease of EBC ezrin after the road exposure session suggests that TRAP exposure might compromise the alveolar epithelial integrity and impair airway epithelial lining, which can further impair lung function and lead to respiratory symptoms.

Our airway metabolomic analysis showed that TRAP exposure was associated with increased metabolites in oxidative stress pathways, including hypoxanthine, uric acid, linoleate, 13-hydroxyoctadecadienoic acid (13-HODE), and methionine. Hypoxanthine was oxidized to xanthine and uric acid by xanthine oxidase, and this process could produce reactive oxygen species (ROS).[49] ROS can further activate lipid oxidation phospholipase A2, which then hydrolyzes phospholipids to polyunsaturated free fatty acids (e.g., linoleic acid).[50] Linoleic acid can be converted into 13-hydroperoxyoctadecadenoic acid

Table 5. Annotated exhaled breath concentrate (EBC) metabolite features associated with high (road)- and low (park)-exposure sessions and with air pollutants in a randomized crossover study of traffic-related air pollution exposure, $n = 56$ adults, Shanghai, China.

m/z	Chemical identity	ESI	Pathway	Estimated associations with TRAP
173.0041	Hypoxanthine	ESI–	Purine metabolism	TRAP (β = 0.028); UFP (/); BC (/); NO_2 (/); CO (/); $PM_{2.5}$ (/)
149.0093	Uric acid	ESI–	Purine metabolism	TRAP (β = 0.030); UFP (/); BC (β = 0.022); NO_2 (/); CO (/); $PM_{2.5}$ (/)
169.0173	Methionine	ESI–	Methionine and cysteine metabolism	TRAP (β = 0.009); UFP (β = 0.008); BC (β = 0.010); NO_2 (/); CO (/); $PM_{2.5}$ (/)
157.1084	L-Arginine	ESI+	Arginine and proline metabolism	TRAP (β = 0.016); UFP (β = 0.011); BC (β = 0.014); NO_2 (β = 0.015); CO (β = 0.012); $PM_{2.5}$ (/)
194.1149	Citrulline	ESI+	Arginine and proline metabolism	TRAP (β = 0.020); UFP (β = 0.015); BC (β = 0.019); NO_2 (β = 0.021); CO (β = 0.017); $PM_{2.5}$ (/)
104.0709	Glutamate	ESI+	Arginine and proline metabolism	TRAP (β = 0.009); UFP (β = 0.006); BC (β = 0.009); NO_2 (β = 0.010); CO (β = 0.013); $PM_{2.5}$ (/)
86.0604	5-Oxoproline	ESI+	Aspartate and asparagine metabolism	TRAP (β = 0.016); UFP (β = 0.011); BC (β = 0.013); NO_2 (β = 0.015); CO (β = 0.014); $PM_{2.5}$ (/)
224.1281	Glutathione	ESI+	Glutathione metabolism; aspartate and asparagine metabolism	TRAP (β = 0.014); UFP (β = 0.012); BC (β = 0.015); NO_2 (β = 0.016); CO (β = 0.013); $PM_{2.5}$ (/)
415.2809	11′-Carboxy-α-tocotrienol	ESI+	Vitamin E metabolism	TRAP (/); UFP (β = 0.015); BC (/); NO_2 (/); CO (β = 0.016); $PM_{2.5}$ (/)
154.1226	13′-Carboxy-α-tocopherol	ESI+	Vitamin E metabolism	TRAP (/); UFP (β = 0.010); BC (/); NO_2 (/); CO (β = 0.010); $PM_{2.5}$ (/)
152.1069	13′-Carboxy-α-tocotrienol	ESI+	Vitamin E metabolism	TRAP (/); UFP (β = 0.025); BC (/); NO_2 (/); CO (β = 0.031); $PM_{2.5}$ (/)
225.0254	L-Lysine	ESI–	Lysine metabolism	TRAP (β = 0.018); UFP (β = 0.014); BC (/); NO_2 (/); CO (/); $PM_{2.5}$ (/)
148.0757	L-Phenylalanine	ESI+	Tyrosine metabolism	TRAP (β = −0.024); UFP (β = 0.014); BC (β = −0.023); NO_2 (β = −0.023); CO (β = −0.020); $PM_{2.5}$ (/)
105.0427	L-Serine	ESI+	Glycine, serine, alanine, and threonine metabolism	TRAP (β = 0.020); UFP (β = 0.019); BC (β = 0.018); NO_2 (β = 0.017); CO (β = 0.020); $PM_{2.5}$ (/)
90.0554	L-Alanine	ESI+	Glycine, serine, alanine, and threonine metabolism	TRAP (β = −0.012); UFP (β = −0.045); BC (β = −0.046); NO_2 (β = −0.012); CO (/); $PM_{2.5}$ (/)
194.9275	Fumarate	ESI–	TCA cycle	TRAP (β = 0.032); UFP (β = 0.025); BC (β = 0.029); NO_2 (/); CO (/); $PM_{2.5}$ (β = 0.006)
117.0189	Succinate	ESI–	TCA cycle	TRAP (β = 0.021); UFP (β = 0.015); BC (β = 0.019); NO_2 (/); CO (/); $PM_{2.5}$ (/)
89.0238	D-Glucose	ESI–	Glycolysis and gluconeogenesis	TRAP (β = 0.012); UFP (β = 0.009); BC (β = 0.011); NO_2 (/); CO (/); $PM_{2.5}$ (/)
91.0215	Lactate	ESI–	Glycolysis and gluconeogenesis	TRAP (β = 0.012); UFP (β = 0.009); BC (β = 0.011); NO_2 (/); CO (/); $PM_{2.5}$ (/)
103.0031	Pyruvate	ESI–	Glycolysis and gluconeogenesis	TRAP (β = 0.018); UFP (β = 0.014); BC (β = 0.017); NO_2 (/); CO (/); $PM_{2.5}$ (/)
351.2212	Leukotriene B_4	ESI–	Leukotriene metabolism	TRAP (/); UFP (β = 0.021); BC (/); NO_2 (/); CO (/); $PM_{2.5}$ (/)
337.237	10,11-Dihydro-leukotriene B_4	ESI–	Leukotriene metabolism	TRAP (/); UFP (β = 0.015); BC (/); NO_2 (/); CO (/); $PM_{2.5}$ (/)
245.226	Linoleate	ESI+	Linoleate metabolism; fatty acid activation	TRAP (β = 0.022); UFP (β = 0.017); BC (β = 0.020); NO_2 (β = 0.026); CO (β = 0.018); $PM_{2.5}$ (/)
168.1131	13-HPODE	ESI+	Linoleate metabolism	TRAP (β = −0.015); UFP (/); BC (/); NO_2 (/); CO (β = −0.014); $PM_{2.5}$ (/)
261.2209	13-HODE	ESI+	Linoleate metabolism	TRAP (β = 0.052); UFP (β = 0.039); BC (β = 0.053); NO_2 (β = 0.061); CO (β = 0.051); $PM_{2.5}$ (/)
259.2034	13-OxoODE	ESI+	Linoleate metabolism	TRAP (β = −0.044); UFP (β = −0.036); BC (β = −0.043); NO_2 (β = −0.036); CO (β = −0.043); $PM_{2.5}$ (/)
301.2159	γ-Linolenic acid	ESI+	Linoleate metabolism; fatty acid activation	TRAP (β = −0.048); UFP(/); BC(/); NO_2 (β = −0.050); CO(/); $PM_{2.5}$ (/)

Note: For the positive-ion mode, the following adducts were considered: M-H_2O+$H^{[1+]}$, M+H_2O+$H^{[1+]}$, M-CO_2+$H^{[1+]}$, M-HCOOK+$H^{[1+]}$, M(C13)+$H^{[1+]}$, M+3$H^{[3+]}$, $M^{[1+]}$, M+$H^{[1+]}$, M+$Na^{[1+]}$, M+H+$Na^{[2+]}$, M-H_4O_2+$H^{[1+]}$. For the negative-ion mode, the following adducts were considered: M+$Cl37^{[-]}$, M(Cl37)-$H^{[-]}$, M-H_2O-$H^{[-]}$, M+Na-2$H^{[-]}$, M+$Br^{[-]}$, M-$H^{[-]}$, M-H+$O^{[-]}$, M-2$H^{[2-]}$, M(C13)-$H^{[-]}$. /, An association (effect) of the metabolites related to the pollutant or TRAPs is not identified in pathway analysis; 13-HODE, 13-hydroxyoctadecadienoic acid; 13-HPODE, 13-hydroperxyoctadecadienoic acid; 13-OxoODE, 13-oxooctadecadienoic acid; BC, black carbon; Br, bromine; CO, carbon monoxide; ESI, electron spray ionization; H, hydrogen; K, potassium; Na, sodium; NO_2, nitrogen dioxide; O, oxygen; $PM_{2.5}$, fine particulate matter; TCA, tricarboxylic acid; TRAP, traffic-related air pollution; UFP, ultrafine particles.

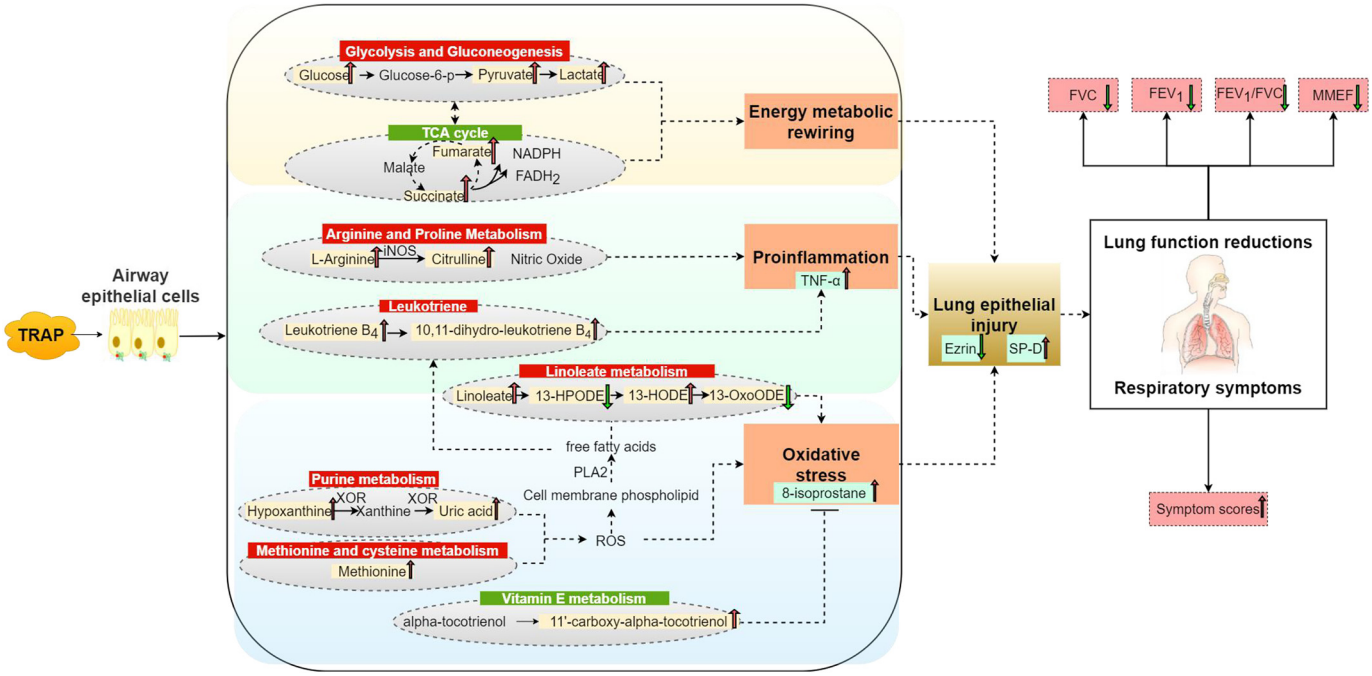

Figure 2. Possible mechanisms underlying the respiratory effects of TRAP exposure identified in this randomized crossover study in China ($n = 56$ adults). Note: 13-HODE, 13-hydroxyoctadecadienoic acid; 13-HPODE, 13-hydroperxyoctadecadienoic acid; 13-OxoODE, 13-oxooctadecadienoic acid; EBC, exhaled breath condensate; $FADH_2$, reduced form of flavin adenine dinucleotide; FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity; iNOS, inducible nitric oxide synthase; MMEF, maximal mid-expiratory flow; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; p, phosphate; PLA2, phospholipase A2; ROS, reactive oxygen species; SP-D, surfactant proteins D; TCA, tricarboxylic acid; TNF-α, tumor necrosis factor-α; TRAP, traffic-related air pollution; XOR, xanthine oxidoreductase.

(13-HPODE) and 13-HODE by lipoxygenases, and the latter is a potential biomarker for oxidative stress-affecting lipids.[50] In this study, we also consistently found that TRAP exposure was associated with higher levels of EBC 8-isoprostane, a biomarker of oxidative stress,[51] further supporting the elevated oxidative stress associated with TRAP exposure.

In addition, our data also linked TRAP exposure with two inflammation-related pathways, the leukotriene metabolism and the arginine and proline metabolism. Consistently, we found higher levels of EBC leukotriene B_4 (a lipid molecule with pro-inflammatory properties) and a slight increase of EBC TNF-α after exposure to high levels of TRAP, indicating that TRAP exposure can increase airway inflammation.[52]

We also observed higher EBC glucose, pyruvate, lactate, succinate, and fumarate after exposure to TRAP. These metabolites are related to energy metabolism, which is essential to cell function and survival. More specifically, the observed increases in these metabolites might suggest up-regulated energy metabolism and activated anaerobic glycolysis in airway epithelial cells. It is possible that energy metabolic rewiring is a potential pathway leading to airway epithelial injury. Consistent with our findings, a recent animal study also found an increased metabolic rearrangement from the TCA cycle to glycolysis as a manifestation of metabolic alteration in lung tissue after $PM_{2.5}$ exposure.[53]

Pollutant-Specific Associations with the Outcomes

We found that $PM_{2.5}$ concentration had a smaller difference between the road and park exposure sessions compared with the other pollutants, which is consistent with previous findings.[18,54] Unlike the other pollutants, $PM_{2.5}$ can be from both local sources and regional transportation.[55] In this study, traffic is the main factor contributing to the differential levels of $PM_{2.5}$ exposure between the road and park sessions. Therefore, when regional transportation dominates, the differences in $PM_{2.5}$ concentrations contributed by the local sources may be masked by the background level.[56]

Among all the pollutants considered in the analysis, NO_2 appeared to have the strongest associations with lung function, especially with decreased MMEF, suggesting NO_2 may impair small airway function. Unlike the other water-soluble gaseous pollutants, NO_2 hydrolyzes slowly in the airway and can reach bronchioles and alveoli and cause mucous edema in the small airways.[57–59] In addition, we found BC and UFP were associated with lung function decline, which was consistent with previous findings in London.[18,19] We found BC and UFP were also associated with changes of biomarkers and metabolic pathways related to oxidative stress and inflammation. Previous studies have reported similar associations of these two pollutants from traffic sources with oxidative stress and inflammation in the respiratory system.[60,61] We did not observe significant associations of $PM_{2.5}$ with any outcome measures, which is in line with prior findings based on the same study design.[18,19] One possible reason for the null associations of $PM_{2.5}$ might be that the difference in $PM_{2.5}$ exposure levels between the road and the park groups was small.

Strengths and Limitations

The randomized crossover trial design of this study can effectively minimize the possibility of unmeasured confounding. Moreover, the combination of the subclinical indicators and the omics technique allowed us to comprehensively investigate the global molecular responses of the respiratory system related to TRAP exposure. Moreover, our metabolomic analysis was based on the EBC samples, which can reflect the local changes in the respiratory tract after exposure to TRAP.

Our study also has several limitations. First, although we controlled for multiple confounders through the randomized crossover study design,[62,63] residual confounding was still possible

under the complex real-life environment. For example, without blinding, participants may differentially report respiratory symptoms between these two exposure sessions. In addition, although we considered only participants who lived on the same campus for at least 1 y to reduce the influence of long-term air pollution exposure, early life air pollution exposure (e.g., exposure during childhood) may have long-term impacts on lung function. Second, all outcome measurements were conducted only once right after each exposure session. Therefore, the delayed effects of TRAP exposure on respiratory health were not considered. Finally, although the effects of the single traffic-related pollutant were examined, our ability to identify the independent effects of each pollutant was limited by the strong correlation (Spearman $r = 0.25–0.87$) among these pollutants. Only healthy young adults were included in this study; therefore, our findings may not be generalizable to other susceptible populations with preexisting conditions.

Conclusion

In this randomized crossover study, we found short-term exposure to TRAP exposure was associated with respiratory symptoms and airway impairment. Airway biomarkers and EBC metabolomic analysis suggested TRAP exposure can lead to lung epithelial injury, airway inflammation, oxidative stress, and energy metabolism disturbance. These findings provide evidence for the adverse respiratory health effects of TRAP exposure and insights into the potential biological mechanisms.

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