Chronic Exposure to Ambient Particulate Matter Induces Gut Microbial Dysbiosis in a Rat COPD Model

Naijian Li
Guangzhou Medical University

Zhaowei Yang
Guangzhou Medical University

Baoling Liao
Guangzhou Medical University

Tianhui Pan
Guangzhou Medical University

Jinding Pu
Guangzhou Medical University

Binwei Hao
Guangzhou Medical University

Zhenli Fu
Guangzhou Medical University

Weitao Cao
Guangzhou Medical University

Yuming Zhou
Guangzhou Medical University

Fang He
Guangzhou Medical University

Bing Li
Guangzhou Medical University

Pixin Ran (✉ pxran@gzhmu.edu.cn)
Guangzhou Institute of Respiratory Health, The State Key Laboratory of Respiratory Disease, The First Affiliated Hospital of Guangzhou Medical University  https://orcid.org/0000-0001-6651-634X

Research

Keywords: Biomass fuel, motor vehicle exhaust, COPD, gut microbiome

DOI: https://doi.org/10.21203/rs.3.rs-19903/v2
Abstract

**Background:** The role of the gut microbiota in the pathogenesis of chronic obstructive pulmonary disease following exposure to ambient particulate matter is largely unknown. We hypothesized that exposure alters gut microbial composition and metabolites and may involve in the pathogenesis of chronic obstructive pulmonary disease.

**Methods:** Fifty-four male Sprague-Dawley rats were exposed to clean air, biomass fuel, or motor vehicle exhaust for 4, 12, and 24 weeks. Lung tissue was assessed histologically and gut microbial composition was assessed by 16S rRNA pyrosequencing. Serum lipopolysaccharide levels were measured and short-chain fatty acids in colon contents were quantified.

**Results:** After a 24-week exposure to particulate matter, rats exhibited pulmonary inflammation and pathological changes characteristic of chronic obstructive pulmonary disease. The gut microbiome was characterized by decreased microbial richness and diversity, distinct overall microbial composition, lower levels of short-chain fatty acids, and higher serum lipopolysaccharide.

**Conclusion:** Chronic exposure to ambient particulate matter induces gut microbial dysbiosis and metabolite shifts in a rat model of chronic obstructive pulmonary disease.

Introduction

Air pollution is a primary environmental cause of chronic respiratory diseases. Industrial activities, vehicular emissions, and household biomass combustion are major sources of ambient particulate matter (PM) [1, 2]. Incomplete combustion of biomass fuel and living in proximity to traffic have been associated with a high prevalence of chronic obstructive pulmonary disease (COPD) [3,4], while reductions in ambient PM have been shown to decrease the risk of COPD [5]. Therefore, it is important to elucidate the mechanism of ambient PM-induced COPD.

In a previous study, we established a rat COPD model via exposure to biomass fuel (BMF) and motor vehicle exhaust (MVE), which can be used to investigate COPD in non-smokers [6]. We found that both BMF and MVE exposure induced airway cells to secrete cytokines that caused pronounced COPD in rats. However, COPD is a complex disease with multiple sub-phenotypes that affects not only the lungs, but also the gastrointestinal tract [7], and its mechanisms are not completely understood. Over the past few years, emerging evidence has suggested that dysbiosis of the gut microbiome may be involved in the pathogenesis of various respiratory diseases [8,9], but the effects of the gut microbiota on COPD following chronic exposure to ambient PM are largely unknown.

Gut exposure to air pollutants occurs via mucociliary transport of PM from the respiratory tract and by ingestion of food and water [10,11], suggesting that the steady-state concentrations of PM in the gut may mirror concentrations in the lung. We hypothesized that chronic exposure to ambient particulate matter induces gut microbial dysbiosis in a rat COPD model. To test our hypothesis, we established a rat COPD
model using exposures to BMF and MVE. Rats were exposed for 4, 12, and 24 weeks, and changes in pulmonary histopathology, microbial composition, and microbial metabolites (lipopolysaccharide [LPS] and short-chain fatty acids [SCFAs]) were measured.

**Methods**

**Animals**

Male Sprague-Dawley rats (7–9 weeks in age) were purchased from Guangzhou University of Chinese Medicine (Guangzhou, China). The Animal Medical Center of Guangzhou Medical University reviewed and approved all experiments (identification number: GY2019-009). A total of 54 rats were randomly divided into three groups: control, BMF, and MVE (n = 6 per group) for three exposure durations (4, 12, and 24 weeks). All rats were kept in a specific pathogen-free room and were housed three to a cage. Except the time of the PM exposure, the animal facility conditions of the BMF and MVE groups are the same as those of the control group. The animal facility maintained temperature and relative humidity at 23 ± 2°C and 40%–70%, respectively. Lighting was artificial with a sequence of 12 h light (06:00–18:00) and 12 h dark. Commercially available rodent food pellets and water were provided ad libitum. Rats were weighed every 2 weeks throughout the study. Corncob bedding and cage are replaced every 3 days. The rats were observed for any sign of illness a minimum of twice daily.

**PM exposure system and characterization of the test atmosphere**

Fig. 1A depicts the design of the study. Rats were exposed to PM as described previously [6]. Briefly, all animals were exposed in whole-body inhalation chambers for 4 h/day, 5 days/week either 4, 12, or 24 weeks. PM mass concentrations, particle size distributions, and gas concentrations were monitored each day of exposure. DustTrak II aerosol monitors (model 8530, TSI, Shoreview, MN, USA) were used to monitored PM mass concentrations and particle size distributions. Testo 340 portable flue gas analyzers (Testo, Lenzkirch, Germany) were used to monitored gas concentrations (O₂, carbon monoxide, nitrogen oxides, and sulfur dioxide) in the exposure rooms. The control group was exposed to clean air.

**Exposure to BMF.** We used China fir sawdust (40 g/per exposure) to produce BMF smoke, which was sent into the animal exposure room through a piston pump (5 L/min). Rats were exposed to BMF smoke for four 1-h periods, 5 days per week. The test atmosphere was measured during the first hour.

**Exposure to MVE.** MVE was produced by a Wuyang model WY48QT-2, 1.6-Kw, 125-cm³, one-cylinder, four-cycle, gasoline-powered motorcycle (Guangzhou, China). Premium low-sulfur gasoline (<150 ppm; Petro Inc., El Paso, TX, USA) was used to produce MVE. Prior to the exposure session, the motorcycle engine was operated in an idle state for 2 min to produce sufficient MVE. Rats were exposed for two 2-h periods, 5 days per week. The test atmosphere was measured during the first 2 h.
Ambient BMF and MVE samples were collected throughout the duration of exposure, and concentrations of organic carbon, elemental carbon, polycyclic aromatic hydrocarbons, and metals were measured at the Guangzhou Institute of Chemistry, Chinese Academy of Sciences (Guangzhou, China) according to a previous method [12, 13].

Measurement of lung function

Spirometry data were obtained as previously described using a Forced Pulmonary Maneuver System (Buxco Research Systems, Wilmington, NC, USA) [6]. Rat were sedated with 3% pentobarbital (1mL/Kg), and were tracheostomized and intubated, then the mice placed supine in the body chamber and connected to the system. According to the procedures, the FRC (functional residual capacity), FEV20 (forced expiratory volume in 20 seconds), FEV100 (forced expiratory volume in 100 seconds) and PEF (peak expiratory flow) were measure. At least three acceptable maneuvers for each test of every mice were conducted to obtain a reliable mean spirometry data.

Sample preparation

Rats were sacriced by CO$_2$ after 4, 12, and 24 weeks of exposure (on days 29, 85, and 169, respectively). Blood samples were collected from the heart and centrifuged at 1,700 × $g$ for 15 min at 4°C. Serum was stored at −80°C. Proximal colon contents were harvested using sterile instruments for each individual animal and site. Fresh proximal colon contents samples were snap-frozen in liquid nitrogen then stored at −80°C for microbial and SCFA analysis.

Bronchoalveolar lavage fluid differential cell count

Bronchoalveolar lavage fluid was collected as previously reported [14]. Cells were isolated by centrifugation at 300 × $g$ for 10 min at 4°C and stained with Diff-Quik stain (Baso Diagnostics, Zhuhai, China). Differential cell counts were assessed from 400 cells counted on each slide.

Lung morphometric analysis

As described previously [6], lung tissues were fixed with 4% paraformaldehyde solution and embedded in paraffin using standard methods. Sectioning and staining were performed by the Pathology Center of the First Affiliated Hospital of Guangzhou Medical University (Guangzhou, China). All slides were scanned and analyzed using an image analyzer platform (Leica, Wetzlar, Germany). Alveolar enlargement and destruction, and the bronchial wall thickness was calculated as describe previous [15].

Serum Levels of Lipopolysaccharide and total BALF protein assay

Serum levels of LPS were measured using a commercial chromogenic end-point TAL kit (Xiamen Bioendo Technology Co., Ltd., Xiamen, China). All procedures were performed according to the manufacturer’s instructions. The total protein in the BALF determined by Bicinchoninic Acid (BCA) method using a commercial BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, USA).
Microbiota analyses

DNA was isolated from colon contents using a Qiagen QIAamp® DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. 16S rRNA gene amplification, in vitro transcription, and labeling and hybridization were performed following the Illumina 16S Metagenomic Sequencing Library Preparation guide [16]. We used a MiSeq rRNA amplicon sequencing protocol to PCR-amplify the V3–V4 variable regions (amplicon size expected: approximately 460 bp). 16S amplicon PCR forward primer was 5'- (TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG)-3' and 16S amplicon PCR reverse primer was 5'- (GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C)-3' [17]. All samples were paired-end sequenced on an Illumina MiSeq PE250 platform (San Diego, CA, USA) by the RiboBio Genome Center (Guangzhou, China). 16S rRNA gene sequence analysis, including raw sequence filtering and taxonomic classification, was performed as described previously [18]. The bar diagrams of alpha diversity indices and relative abundance were drawn using GraphPad Prism 8 software (La Jolla, CA, USA).

Quantification of SCFAs in colon contents

Seven SCFAs (acetic, propionic, butyric, isobutyric, valeric, isovaleric, and caproic acids) in the colon were measured by high-performance gas chromatography-mass spectrometry (Agilent 6890N; Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's recommendations. Briefly, a total of 100 mg of the colon contents were homogenized by ultrasonication in 600-μL reactions containing 100 μL of phosphoric acid (15%), 100 μL of 4-methylvaleric acid (250 μg/mL), and 400 μL of diethyl ether. The mixtures were vortexed and centrifuged until a clear supernatant was obtained. The sample was subjected to a high-performance liquid chromatography column (Agilent Technologies) for analysis of SCFAs.

Statistical analysis

Data are reported as mean ± standard deviation (SD). Comparisons were performed using ANOVA and p values were corrected for multiple testing with the Bonferroni method. Statistical analysis was performed using SPSS version 24 (IBM SPSS, Armonk, NY, USA). Correlations between serum LPS levels and the pulmonary mean linear intercept were assessed using Spearman's rank correlation. p < 0.05 was considered significant.

Results

Particle size and gas concentrations during PM exposure

Particle size distributions and gas concentrations were measured during PM exposure. Fig. 2 and Table 1 show the concentrations of PM with a diameter ≤10, 2.5, and 1 μm (PM_{10}, PM_{2.5}, and PM_{1}) during BMF and MVE exposures. Average concentrations of PM_{10}, PM_{2.5}, and PM_{1} in the BMF exposure were 25.87 ± 2.99 mg/m^3, 21.91 ± 1.84 mg/m^3, and 19.60 ± 1.76 mg/m^3, respectively. In the MVE exposure, average
concentrations were 1.86 ± 0.20 mg/m$^3$, 1.85 ± 0.24 mg/m$^3$, and 1.82 ± 0.27 mg/m$^3$, respectively. BMF exposure caused notably higher particulate emissions. To estimate levels of gaseous pollutants generated by combustion, we also measured gas concentrations during both BMF and MVE exposures. Table 1 lists the mean ± SD concentrations of O$_2$, carbon monoxide, nitrogen oxides, and sulfur dioxide during exposure. Tables 2–4 list the elemental composition of the particulates.

**Evaluation of COPD model**

Exposure to PM affected body weight and induced pulmonary inflammation. Rats continued to gain weight during PM exposure, with a total weight gain of 162.3% (controls), 135.2% (BMF group), and 130.4% (MVE group). The BMF and MVE groups maintained a steady rate of weight gain throughout the exposure period, but the rate was slower than the rate of weight gain in the controls after 4 weeks ($p < 0.01$; Fig. 2A). The rats exposed to BMF or MVE had more sediment particulate matter in bronchoalveolar lavage fluid as early as 4 weeks into the exposure period (Fig. 2B). The levels of total protein in BALF were both higher in the MVE group or BMF group than in controls since a 4-week exposure ($p < 0.05$ or $p < 0.01$, Fig. 2C). Moreover, a significant increase in total leukocyte counts were observed both in the BMF group and MVE group ($p < 0.05$ or $p < 0.01$, Fig. 2D). The total leukocyte counts started to increase greatly since a 4-week exposure and remained elevated to the 24 weeks of exposure. Differential cell counts showing that increase in total leukocyte counts were due mainly to increases in alveolar macrophages and neutrophils counts ($p < 0.05$ or $p < 0.01$, Fig. 2E-F), and the alveolar macrophages have the highest percentage in total leukocyte counts.

Exposure to PM also induced COPD-like changes in rat lung. Histological analysis showed that significant increases in the mean linear intercept after 24 weeks of exposure in both the BMF ($p < 0.01$) and MVE ($p < 0.01$) groups compared to controls (Fig. 3A). Long-term PM exposure can be damaged the lung parenchyma. After 24 weeks BMF and MVE particles exposure, the thickness of bronchial walls in the lungs of rats was increase greatly to that of controls (Fig. 3B), suggesting that the PM exposures induced airway remodeling. Moreover, after 24-weeks of exposure, the pulmonary function test results of FRC were significantly increased, but the PEF, FEV20/FVC and FEV100 were decreased significantly both in the BMF group and MVE group than from controls ($p < 0.01$, Fig. 3C). Together, these findings suggest that chronic exposure to BMF and MVE induced COPD-like changes in the rat lung.

**Gut microbial diversity**

To determine whether chronic exposure to ambient PM induces intestinal microbial shifts, we performed 16S rRNA gene sequencing of colon contents from controls and rats exposed to BMF or MVE after 4, 12, and 24 weeks of exposure. After filtering for low-quality reads, 4,479,175 sequence reads from 54 samples were used for analysis and resulted in 22,000 operational taxonomic units. Comparisons between the three groups showed that intra-individual diversity, as measured by the number of operational taxonomic units, decreased significantly in the MVE group after 24 weeks of exposure ($p = 0.062$, vs CON group; $p = 0.122$, vs BMF group, Fig. 4A). Other indices (Chao 1, PD_whole_tree, and the
Shannon index) were calculated to estimate the within-sample (alpha) diversity. The Chao 1 \((p = 0.090, \text{ vs CON group; Fig. 4 B})\) and PD\_whole\_tree indices \((p = 0.216, \text{ vs CON group, Fig. 4 C})\) were lower in the MVE group after 24 weeks exposure. The reduced richness of operational taxonomic units and alpha diversity in the gut microbiota suggest a possible deficiency in healthy microflora in the BMF and MVE groups.

To investigate the difference between gut microbial communities in the three groups, we analyzed the taxonomical community structure of the microbiome in colon contents. At the phylum level, all samples at all measurement time points from the three groups contained four major bacterial phyla: Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria. The first three phyla accounted for over 97% of the total sequences in all three groups (Fig. 5). After a 4-week exposure, the MVE group had a lower relative abundance of Firmicutes \((p = 0.003 \text{ vs. the control group}; p = 0.002 \text{ vs. the BMF group})\) but a higher relative abundance of Proteobacteria \((p = 0.099 \text{ vs. the control group}; p = 0.055 \text{ vs. the BMF group, Fig. 5A})\). After a 12-week exposure, the MVE group exhibited relative abundances of Firmicutes \((p = 0.019 \text{ vs. the control group}; p = 0.072 \text{ vs. the BMF group, Fig. 5B})\) that were consistent with the trend seen after 4 weeks of exposure. However, after 24 weeks of exposure, the three groups exhibited a similar relative abundance of four major bacterial phyla \((p > 0.05, \text{ Fig. 5C})\). These findings indicate gut microbiota dysbiosis after PM exposure.

**Quantification of microbial metabolites**

We analyzed the levels of SCFAs in colon contents following 4- and 24-week exposures using gas chromatography. Acetic, propionic, and caproic acids were detected in all colon contents, but butyric, isobutyric, valeric, and isovaleric acids were not detected in some samples. SCFA levels differed between the control and exposure groups. Levels of total SCFAs were significantly lower in the MVE group than in controls \((p = 0.125)\) after 4 weeks and 24-week exposure \((p = 0.041, \text{ Fig. 6A})\). Moreover, a significant decrease in acetic acid was observed in colon contents from the MVE group relative to contents in controls \((p = 0.036, \text{ Fig. 6B})\). Propionic and caproic acids exhibited similar levels after PM exposure (Fig. 6C, D).

Serum LPS levels were higher in the MVE group than in controls after a 4-week exposure \((p = 0.054)\). After a 24-week exposure, serum LPS levels were significantly higher in both the BMF \((p = 0.007)\) and MVE \((p = 0.007)\) groups than in controls (Fig. 6E), and exhibited a weak positive correlation with the pulmonary mean linear intercept \((r^2 = 0.4552, p < 0.001; \text{ Fig. 6F})\).

**Discussion**

In recent years, the association of ambient PM exposure with COPD has been well-established [19]. In previous work, we found that elevated PM concentrations were associated with increased COPD prevalence and diminished respiratory function [4]. Moreover, use of clean fuels and improved ventilation have been associated with better pulmonary function and a reduced risk of COPD [5]. We then established a rat COPD model using exposure to biomass fuel and motor vehicle exhaust to study the
pathophysiology of PM-induced COPD [6], but the underlying biological mechanisms are still unclear. PM research has mostly focused on the lung inflammatory response to inhalation, endothelial dysfunction, and oxidative stress, but the effects of PM on the intestine and its role in the pathogenesis of COPD are largely unknown. In the present study, we demonstrated that chronic exposure to PM induced COPD-like characteristic pathological changes and pulmonary inflammation in rats that were concomitant with a decrease in the abundance and diversity of gut microbiota.

Recent studies have suggested a role for the gut microbiota in the development of respiratory diseases such as asthma, pneumonia, and acute respiratory distress syndrome [9,20,21], although the connection between the lung and the gut is poorly understood. In the present study, we found that PM exposure was associated with decreased abundance and diversity in bacterial populations, similar to the effects reported for fine PM on diabetes and obesity [22-24]. These data from epidemiological and animal studies suggest that long-term exposure to PM causes gut microbiota dysbiosis and may subsequently contribute to increased risk of diabetes and obesity [22,23]. Importantly, a chronic exposure (24 weeks) to PM reduced operational taxonomic units and alpha diversity, consistent with the findings of a study conducted in mice [22]. In addition to diversity, bacterial relative abundance was also affected, and may partially mediate the association of PM with COPD. Notably, the abundance of Firmicutes was first reduced in rats exposed to MVE group, but after 24 weeks of exposure, the relative abundance of Firmicutes increased from 80% to 97%, at the same time that the relative abundance of Firmicutes in rats exposed to BMF decreased from 97% to 88%. Our findings show that gut microbiota dysbiosis in a PM-induced rat model of COPD may depend on exposure duration, the dose, or the sources of PM.

Despite the well-studied pulmonary effects of ambient PM, little is known about its effects on the gastrointestinal tract and microbiome. Ambient PM can enter gastrointestinal tract through the bloodstream or by ingestion, either after clearance via airway cilia or by consumption of contaminated food and water [10,11]. Salim et al. and Beamish et al. proposed a model of PM-induced intestinal and systemic inflammation, suggesting that PM in the gastrointestinal tract causes oxidative stress and increases gut permeability [25-27]. This enables microbial products and PM to penetrate the lamina propria and interact with immune cells. Inflammatory responses in intestinal cells will further alter the micro-environment and enable the growth of particular microbial strains. The altered microbial community and its products affect metabolic processes and thus become a causative factor in disease progression. This imbalance in gut microbiota leads to the migration of bacteria or bacterial products, such as LPS, from the intestinal lumen to mesenteric lymph nodes or the circulation, in association with an inflammatory response [28-30]. LPS is released during bacterial cell division or death and is pro-inflammatory [31, 32]. We found that serum LPS levels increased after both BMF and MVE exposures. LPS causes the production of inflammatory mediators, and the process can result in pathological changes characteristic of COPD in the lung [33].

SCFAs are metabolized by the gut microbiota from otherwise indigestible fiber-rich foods and are involved in metabolism [34]. SCFAs have anti-inflammatory properties, are a source of energy for colonocytes, improve gut barrier function, and reduce intestinal bacterial translocation [35]. A high-fiber diet has been
suggested to decrease COPD risk, primarily in current and former smokers [36,37]. Our findings suggest that the decrease in SCFA levels observed following exposure to BMF or MVE. Animal experiments also support this SCFA-related mechanism; an increase in dietary SCFAs was reported to attenuate the development of smoking-related emphysema in rats and mice [38,39]. We propose that SCFAs, and especially acetic acid, may confer a protective effect against PM-induced COPD.

Although the study demonstrates a notable impact of inhaled PM on the gut microbiota and implicates it in the development of COPD, several limitations should be noted. First, in the absence of interventional research, it is difficult to ascertain whether gut microbiota dysbiosis is a cause or a consequence of COPD. This will require the use of germ-free mice and gut microbial transplantation. Second, although exposure to PM was shown to alter the composition and function of gut microbiota, how PM inhalation affects the gut microbiota remains to be determined. More studies are warranted to better understand the pathogenesis of gut microbiota dysbiosis after chronic exposure to ambient PM. Third, we cannot yet ascertain whether PM induction of gut microbiota dysbiosis is time- or dose-dependent.

**Conclusions**

Chronic exposure to ambient PM decreases the levels of SCFAs in the colon and induces gut microbial shifts and translocation in a rat COPD model.

**Declarations**

**Ethics approval**

All animal care and experiment protocols complied with the guiding principles for the care and use of laboratory animals recommended by the Chinese Association for Laboratory Animal Science Policy. The Animal Medical Center of Guangzhou Medical University reviewed and approved all experiments (identification number: GY2019-009).

**Consent for publication**

Not applicable.

**Availability of data and material**

Please contact author for data requests.

**Funding**

This work was supported by a grant from the National Program on Key Basic Research Projects (2015CB553403), Youth Foundation of the National Key Laboratory of Respiratory Diseases (SKLRD-QN-201908), Science and Technology Program of Guangzhou (201504010018), National Natural Science Foundation of China (81670040, 81570035, 81970045, and 81900030), Local Innovative and Research
Teams Project of Guangdong Pearl River Talents Program (2017BT01S155), and 135 Key Research and Development Program (2016YFC1304101).

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

NJL, PXR, YMZ, and BL conceived the study, directed the project, and designed the experiments; THP, BLL, BWH, WTC, ZLF, FH, and JDP obtained the samples, interpreted the results, and analyzed the animal data; NJL and ZWY performed the microbial sequencing analysis and wrote the manuscript; all authors read and approved the final manuscript.

Acknowledgements

We thank Liwen Bianji, Edanz Group China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.

Author details

1Department of Allergy and Clinical Immunology, State Key Laboratory of Respiratory Disease, National Clinical Research Center for Respiratory Disease, Guangzhou Institute of Respiratory Health, the First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510000, P.R. China

2Nanhai Hospital Southern Medical University, Foshan 528000, P.R. China

3State Key Laboratory of Respiratory Disease, National Clinical Research Center for Respiratory Disease, Guangzhou Institute of Respiratory Health, the First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510000, P.R. China

4Department of Respiratory Medicine, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou 510000, P.R. China

5The School of Basic Medicine, Guangzhou Medical University, Guangzhou 510000, P.R. China

6The GMU-GIBH Joint School of Life Sciences, Guangzhou Medical University, Guangzhou 510000, P.R. China

Abbreviations

BMF, biomass fuel; COPD, chronic obstructive pulmonary disease; FEV20, forced expiratory volume in 20 seconds; FEV100, forced expiratory volume in 100 seconds; FRC, functional residual capacity; LPS,
lipopolysaccharide; MVE, motor vehicle exhaust; PEF, peak expiratory flow; PM, particulate matter; SCFA, short-chain fatty acid.

References

1. Guan WJ, Zheng XY, Chung KF, Zhong NS. Impact of air pollution on the burden of chronic respiratory diseases in China: time for urgent action. Lancet. 2016;15;388(10054):1939-1951.

2. To T, Zhu J, Larsen K, Simatovic J, Feldman L, Ryckman K, Gershon A, Lougheed MD, Licskai C, Chen H, Villeneuve PJ, Crighton E, Su Y, Sadatsafavi M, Williams D, Carlsten C. Progression from Asthma to Chronic Obstructive Pulmonary Disease. Is Air Pollution a Risk Factor? Am J Respir Crit Care Med. 2016;15;194(4):429-438.

3. Zeki AA, Flayer CH, Haczku A. A burning need to redefine airways disease: Biomass smoke exposure identified as a unique risk factor for asthma-chronic obstructive pulmonary disease overlap in low- and middle-income countries. J Allergy Clin Immunol. 2019;143(4):1339-1341.

4. Liu S, Zhou Y, Liu S, Chen X, Zou W, Zhao D, Li X, Pu J, Huang L, Chen J, Li B, Liu S, Ran P. Association between exposure to ambient particulate matter and chronic obstructive pulmonary disease: results from a cross-sectional study in China. Thorax 2016;0:1-8.

5. Zhou Y, Zou Y, Li X, Chen S, Zhao Z, He F, Zou W, Luo Q, Li W, Pan Y, Deng X, Wang X, Qiu R, Liu S, Zheng J, Zhong N, Ran P. Lung function and incidence of chronic obstructive pulmonary disease after improved cooking fuels and kitchen ventilation: a 9-year prospective cohort study. PLoS Med. 2014;25;11(3):e1001621.

6. He F, Liao B, Pu J, Li C, Zheng M, Huang L, Zhou Y, Zhao D, Li B, Ran P. Exposure to ambient particulate matter induced COPD in a rat model and a description of the underlying mechanism. Sci Rep. 2017;31;7: 45666.

7. Young RP, Hopkins RJ, Marsland B. The Gut-Liver-Lung Axis. Modulation of the Innate Immune Response and Its Possible Role in Chronic Obstructive Pulmonary Disease. Am J Respir Cell Mol Biol. 2016;54(2) :161-169.

8. Chakradhar S. A curious connection: Teasing apart the link between gut microbes and lung disease. Nat Med. 2017;23(4):402-404.

9. Dickson RP, Singer BH, Newstead MW, et al. Enrichment of the Lung Microbiome with Gut Bacteria in Sepsis and the Acute Respiratory Distress Syndrome. Nat Microbiol. 2016;18;1(10):16113.

10. Alpofead JAH, Davidson CM, Littlejohn D. A novel two-step sequential bioaccessibility test for potentially toxic elements in inhaled particulate matter transported into the gastrointestinal tract by mucociliary clearance. Anal Bioanal Chem. 2017;409(12):3165-3174.

11. De Brouwere K, Buekers J, Cornelis C, Schlekat CE, Oller AR. Assessment of indirect human exposure to environmental sources of nickel: oral exposure and risk characterization for systemic effects. Sci Total Environ. 2012;1;419:25-36.
12. Wang X, Chen M, Zhong M, Hu Z, Qiu L, Rajagopalan S, et al. Exposure to Concentrated Ambient PM2.5 Shortens Lifespan and Induces Inflammation-Associated Signaling and Oxidative Stress in Drosophila. Toxicological sciences : an official journal of the Society of Toxicology. 2017;156:199-207.

13. Wu SP, Tao S, Zhang ZH, Lan T, Zuo Q, et al. Distribution of particle-phase hydrocarbons, PAHs and OCPs in Tianjin, China. Atmos Environ, 2005,39:7420-7432.

14. Li N, He F, Liao B, Zhou Y, Li B, Ran P, et al. Exposure to ambient particulate matter alters the microbial composition and induces immune changes in rat lung. Respir Res. 2017;18(1):143.

15. Hsia CC, Hyde DM, Ochs M, et al. An official research policy statement of the American Thoracic Society/European Respiratory Society: standards for quantitative assessment of lung structure. Am J Respir Crit Care Med.2010;181(4):394-418.

16. 16S Metagenomic Sequencing Library Preparation. San Diego, CA: Illumina. Updated 2014. Retrieved July 15, 2016, from https://www.illumina.com/.

17. Klindworth A1, Pruesse E, Schweer T, Peplies J, Quast C, Glöckner FO, et al. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 2013;41(1):e1.

18. Rocafort M, Noguera-Julian M, Rivera J, Pastor L, Guillén Y, Langhorst J, et al. Evolution of the gut microbiome following acute HIV-1 infection. Microbiome.2019;11;7(1):73.

19. Siddharthan T, Grigsby MR, Goodman D, et al. Association between Household Air Pollution Exposure and Chronic Obstructive Pulmonary Disease Outcomes in 13 Low- and Middle-Income Country Settings. Am J Respir Crit Care Med.2018;197(5):611-620.

20. Fujimura KE, Lynch SV. Microbiota in allergy and asthma and the emerging relationship with the gut microbiome. Cell Host Microbe.2015;17(5):592-602.

21. Tamburini S, Clemente JC. Neonatal gut microbiota induces lung immunity against pneumonia. Nat Rev Gastroenterol Hepatol. 2017;14(5):263-264.

22. Wang W, Zhou J, Chen M, et al. Exposure to concentrated ambient PM2.5 alters the composition of gut microbiota in a murine model. Part Fibre Toxicol.2018;15(1):17.

23. Alderete TL, Jones RB, Chen Z, et al. Exposure to Traffic-Related Air Pollution and the Composition of the Gut Microbiota in Overweight and Obese Adolescents. Environ Res.2018;161:472-478.

24. Liu T, Chen X, Xu Y, et al. Gut microbiota partially mediates the effects of fine particulate matter on type 2 diabetes: Evidence from a population-based epidemiological study. Environ Int. 2019;130:104882.

25. Salim SY, Kaplan GG, Madsen KL. Air pollution effects on the gut microbiota: a link between exposure and inflammatory disease. Gut Microbes.2014;5(2):215-219.

26. Beamish LA, Osornio-Vargas AR, Wine E. Air pollution: An environmental factor contributing to intestinal disease. J Crohns Colitis.2011;5(4):279-286.
27. Mutlu EA, Engen PA, Soberanes S, et al. Particulate matter air pollution causes oxidant-mediated increase in gut permeability in mice. Part Fibre Toxicol. 2011;8:19.

28. Cani PD, Jordan BF. Gut microbiota-mediated inflammation in obesity: a link with gastrointestinal cancer. Nat Rev Gastroenterol Hepatol. 2018;15(11):671-682.

29. Cox AJ, West NP, Cripps AW. Obesity, inflammation, and the gut microbiota. Lancet Diabetes Endocrinol. 2015;3(3) :207-215.

30. Perry RJ, Camporez JG, Kursawe R, et al. Hepatic acetyl CoA links adipose tissue inflammation to hepatic insulin resistance and type 2 diabetes. Cell. 2015; 160 (4):745-758.

31. Feehley T, Belda-Ferre P, Nagler CR, et al. What’s LPS Got to Do with It? A Role for Gut LPS Variants in Driving Autoimmune and Allergic Disease. Cell Host Microbe. 2016;19(5) :572-4.

32. Stevens BR, Goel R, Seungbum K, et al. Increased human intestinal barrier permeability plasma biomarkers zonulin and FABP2 correlated with plasma LPS and altered gut microbiome in anxiety or depression. Gut. 2018;67(8) :1555-1557.

33. Korsgren M, Linden M, Entwistle N, et al. Inhalation of LPS induces inflammatory airway responses mimicking characteristics of chronic obstructive pulmonary disease. Clin Physiol Funct Imaging. 2012;32(1):71–79.

34. Lau WL, Vaziri ND. Gut microbial short-chain fatty acids and the risk of diabetes. Nat Rev Nephrol. 2019;15(7):389-390.

35. Sanna S, van Zuydam NR, Mahajan A, et al. Causal relationships between gut microbiome, short-chain fatty acids and metabolic diseases. Nat Genet. 2019;51(4): 600–605.

36. Kaluza J, Harris H, Wallin A, et al. Dietary Fiber Intake and Risk of Chronic Obstructive Pulmonary Disease: A Prospective Cohort Study of Men. Epidemiology. 2018;29(2):254-260.

37. Varraso R, Chiuve SE, Fung TT, Barr RG, Hu FB, Willett WC. Alternate Healthy Eating Index 2010 and risk of chronic obstructive pulmonary disease among US women and men: prospective study. BMJ. 2015;350:h286.

38. Tomoda K, Kubo K, Yamamoto Y, et al. Alteration in gut environment accelerates emphysematous lesions by cigarette smoke in rats discontinuously fed with a fiber-free diet. Am J Respir Crit Care Med. 2014;189:A3000.

39. Tomoda K, Kubo K, Dairiki K. et al. Whey peptide-based enteral diet attenuated elastase-induced emphysema with increase in short chain fatty acids in mice. BMC Pulm Med. 2015,10;15:64.

**Table**

**Table 1. Concentrations of particulate matter (PM) and gaseous pollutants measured during exposure**
| Test items          | CON   | BMF       | MVE       |
|---------------------|-------|-----------|-----------|
| PM10 (mg/m³)        | —     | 25.87±2.99| 1.86±0.20 |
| PM2.5 (mg/m³)       | —     | 21.91±1.84| 1.85±0.24 |
| PM1 (mg/m³)         | —     | 19.60±1.76| 1.82±0.27 |
| NO₁ (ppm)           | —     | 0.78 ± 0.10| 0.31 ± 0.07|
| NOₓ (ppm)           | —     | 0.78 ± 0.10| 0.31 ± 0.07|
| SO₂ (ppm)           | —     | —         | 0.82 ± 0.10|
| CO (ppm)            | —     | 110.1±15.4| 129.0±12.6|
| O₂ (%)              | 20.9 ± 0.04| 20.7±0.06| 20.4±0.05 |
| Humidity (%)        | 60.1±5.6| 66.0±9.3  | 73.5±9.9  |
| Temperature (°C)    | 24.9±0.5| 26.1±1.8  | 27.5±4.4  |

Values are shown as mean ± SD. CON, control group; BMF, biomass fuel exposure group; MVE, motor vehicle exhaust exposure group.

Table 2. The elemental composition of PAHs in ambient BMF and MVE samples.

| PAHs                   | BMF (mg/kg) | MVE (mg/kg) |
|------------------------|-------------|-------------|
| Naphthalene            | 6.95 ± 2.24 | —           |
| Acenaphthylene         | —           | 0.03 ± 0.03 |
| Acenaphthene           | —           | —           |
| Fluorene               | —           | 0.06 ± 0.05 |
| Phenanthrene           | 2.34 ± 1.07 | 2.22 ± 1.30 |
| Anthracene             | 0.20 ± 0.05 | 0.07 ± 0.03 |
| Fluoranethene          | 1.04 ± 0.28 | 4.17 ± 3.16 |
| Pyrene                 | 0.16 ± 0.13 | 7.71 ± 5.80 |
| Benzo anthracene       | 0.22 ± 0.19 | 2.25 ± 1.24 |
| Chrysene               | —           | 1.05 ± 0.73 |
| Benzo[b+j] fluoranthene| —           | 4.02 ± 3.90 |
| Benzo[k] fluoranthene  | —           | 0.59 ± 0.38 |
| Benzo[a] pyrene        | —           | 2.19 ± 1.70 |
| Benzo[e] pyrene        | —           | 2.69 ± 1.30 |
| Dibenz[a,h] anthracene | —           | —           |
| Benzo[g,h,i] perylene  | —           | —           |
| Indeno[1,2,3-cd] pyrene| —           | 4.81 ± 2.75 |

Results are expressed as mean ± SD; Concentrations were expressed as mg/kg; PAHs, Polycyclic Aromatic Hydrocarbons; Horizontal line mean below detection limit; BMF, biomass fuel exposure group; MVE, motor vehicle exhaust exposure group.
Table 3. The elemental composition of OC/EC in ambient BMF and MVE samples.

| OC/EC | BMF (ng/ug) | MVE (ng/ug) |
|-------|-------------|-------------|
| TC    | 117.24 ± 3.39 | 153 ± 21.66 |
| OC    | 116.70 ± 3.31 | 146.11 ± 19.79 |
| EC    | 0.54 ± 0.08 | 6.89 ± 1.87 |
| OC/EC | 28.10 ± 1.98 | 10.72 ± 2.11 |
| OC1   | 67.33 ± 0.63 | 36.72 ± 7.67 |
| OC2   | 26.50 ± 1.13 | 102.09 ± 11.62 |
| OC3   | 6.86 ± 0.37 | 4.97 ± 0.38 |
| OC4   | 1.03 ± 0.18 | 1.36 ± 0.46 |
| EC1   | 15.4 ± 1.05 | 7.79 ± 1.48 |
| EC2   | 0.08 ± 0.02 | 0.08 ± 0.04 |
| EC3   | 0.05 ± 0.02 | — |
| OP2   | 14.99 ± 1.02 | 0.97 ± 0.34 |

Results are expressed as mean ± SD; OC/EC, organic carbon/elemental carbon; Horizontal line mean below detection limit; BMF, biomass fuel exposure group; MVE, motor vehicle exhaust exposure group.

Table 4. The metal composition in ambient BMF and MVE samples.

| Metal | BMF (ng/ug) | MVE (ng/ug) |
|-------|-------------|-------------|
| Na    | 0.003 ±0.003 | 0.16 ± 0.09 |
| Mg    | 0.01 ±0.01  | 0.11 ± 0.11 |
| Al    | —           | —           |
| Si    | —           | 0.04 ± 0.03 |
| S     | 0.15 ± 0.10 | 1.13 ± 0.26 |
| Cl    | 0.20 ± 0.15 | 0.03 ± 0.03 |
| K     | 0.002 ± 0.001 | 0.03 ± 0.01 |
| Ca    | —           | 0.52 ± 0.13 |
| Ti    | —           | 0.002 ± 0.002 |
| V     | —           | —           |
| Cr    | —           | —           |
| Mn    | 0.001 ± 0.001 | 0.002 ± 0.001 |
| Fe    | —           | 0.27 ± 0.10 |
| Co    | —           | —           |
| Ni    | —           | —           |
| Cu    | —           | 0.01 ± 0.01 |
| Zn    | —           | 0.30 ± 0.14 |
| Rb    | —           | —           |
| Sb    | 0.002 ± 0.001 | — |
| Ba    | 0.004 ± 0.002 | 0.02 ± 0.01 |
| Pb    | —           | 0.01 ± 0.01 |

Results are expressed as mean ± SD; Horizontal line mean below detection limit; BMF, biomass fuel exposure group; MVE, motor vehicle exhaust exposure group.

Figures
Figure 1

Schematic overview of the study workflow. (A) A total of 54 rats were randomly divided into three groups (control [CON], biomass fuel [BMF], and motor vehicle exhaust [MVE]; n = 6 per group) and exposed for 4, 12, or 24 weeks. Lung tissue was assessed histologically and gut microbial composition was assessed by 16S rRNA pyrosequencing. Serum lipopolysaccharide (LPS) levels were measured and short-chain fatty acids (SCFAs) in colon contents were quantified. (B) Particulate matter (PM) concentrations and particle size distributions during exposure. Rats exposed to biomass fuel (BMF) inhaled higher concentrations of PM with a diameter $\leq 10$, 2.5, and 1 $\mu$m (PM10, PM2.5, and PM1) than rats exposed to motor vehicle exhaust (MVE). In the BMF group, PM10 concentrations were highest; concentrations of PM10, PM2.5, and PM1 did not differ in the MVE group. Boxes and the inside line represent the mean ± SD for PM2.5.
Figure 2

Exposure to biomass fuel (BMF) and motor vehicle exhaust (MVE) induces pulmonary inflammation in rats. (A) Effects of BMF and MVE exposure on body weight. (B) Particle sediments were observed in bronchoalveolar lavage fluid in the BMF and MVE groups during the exposure period (n = 6). (C) The levels of total protein in BALF. (D) BALF total leukocyte counts. (E-F) BALF alveolar macrophages counts and neutrophil counts. n = 6; *p < 0.05, **p < 0.01. CON, control group.
Exposure to biomass fuel (BMF) and motor vehicle exhaust (MVE) induces effects consistent with chronic obstructive pulmonary disease in rats. (A) Lung sections show significant increases in the mean linear intercept after 24 weeks of BMF and MVE exposure. (B) The thickness of the small airway wall increased significantly in the rats after 24 weeks of BMF and MVE exposure. (C) Effects of BMF and MVE exposure on rat pulmonary function test results. n = 6. **p < 0.01. CON, control group.
Figure 4

Microbial abundance and diversity following exposure to biomass fuel or motor vehicle exhaust. Comparison of the operational taxonomic units (OTUs) (A) and alpha diversity (as assessed by the Chao 1 [B] and PD_whole_tree [C] indices; n = 6 per group). CON, control group.

Figure 5

Relative proportions of major bacterial phyla following exposure to particulate matter. Microbial abundance was measured in colon contents from rats exposed to clean air (CON) and to particulate matter from biomass fuel (BMF) or motor vehicle exhaust (MVE).
Figure 6

Microbial metabolites in colon contents of controls (CON) and rats exposed to biomass fuel (BMF) or motor vehicle exhaust (MVE). (A) Levels of total short-chain fatty acids (SCFAs) were lower in the MVE groups after 24 weeks of exposure. (B) Levels of acetic acid were lower in the MVE groups, especially after 24 weeks of exposure. (C-D) Levels of propionic acid and caproic acid did not differ between groups. (E) Serum lipopolysaccharide (LPS) levels in the three groups after 4 and 24 weeks of exposure. (F) Elevated serum LPS levels were correlated with the mean linear intercept. Boxes and the inside line represent the mean ± SD; each dot corresponds to a sample. n = 6. *p < 0.05, **p < 0.01.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable.pdf