Report

www.the-innovation.org

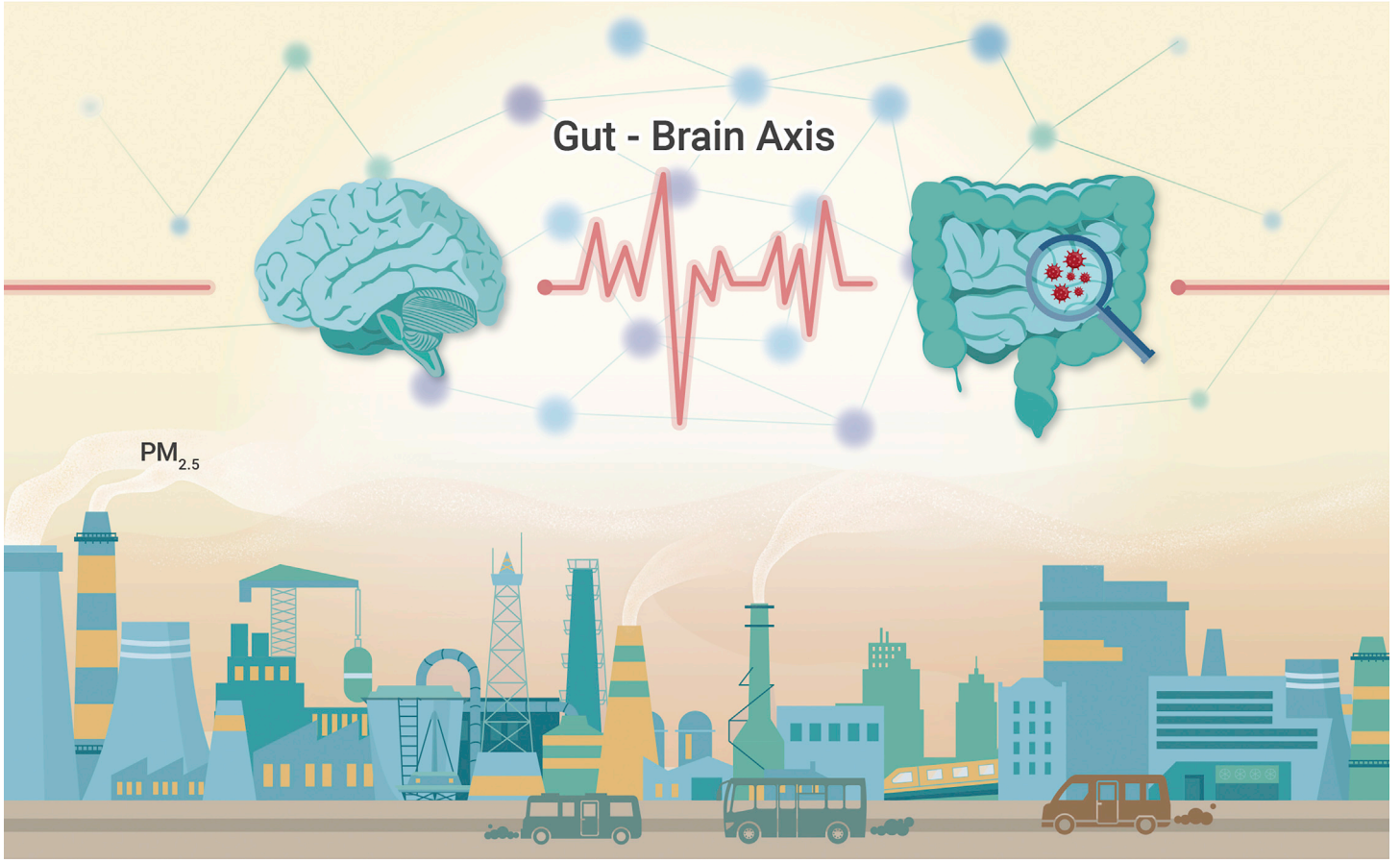
$PM_{2.5}$ exposure associated with microbiota gut-brain axis: Multi-omics mechanistic implications from the BAPE study

Tiantian Li,[1,4] Jianlong Fang,[1,4] Song Tang,[1,4] Hang Du,[1,4] Liang Zhao,[1] Yanwen Wang,[1] Fuchang Deng,[1] Yuanyuan Liu,[1] Yanjun Du,[1] Liangliang Cui,[2] Wanying Shi,[1] Yan Wang,[3] Jiaonan Wang,[1] Yingjian Zhang,[2] Xiaoyan Dong,[1] Ying Gao,[1] Yu Shen,[1] Li Dong,[1] Huichan Zhou,[1] Qinghua Sun,[1] Haoran Dong,[1] Xiumiao Peng,[2] Yi Zhang,[1] Meng Cao,[2] Hong Zhi,[2] Jingyang Zhou,[3] and Xiaoming Shi[1,*]

*Correspondence: shixm@chinacdc.cn

Received: August 7, 2021; Accepted: January 26, 2022; Published online: February 3, 2022; https://doi.org/10.1016/j.xinn.2022.100213

GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- This is a real-world population based panel study using multi-omics technology
- Link between $PM_{2.5}$ and microbiota gut-brain axis is reported for the first time
- $PM_{2.5}$ affected gut microbiota, tryptophan metabolism, and inflammatory factors
- Important hormones of the HPA axis increased with $PM_{2.5}$ exposure
- $PM_{2.5}$ was associated with nervous and cardiovascular outcomes

Report

$PM_{2.5}$ exposure associated with microbiota gut-brain axis: Multi-omics mechanistic implications from the BAPE study

Tiantian Li,[1,4] Jianlong Fang,[1,4] Song Tang,[1,4] Hang Du,[1,4] Liang Zhao,[1] Yanwen Wang,[1] Fuchang Deng,[1] Yuanyuan Liu,[1] Yanjun Du,[1] Liangliang Cui,[2] Wanying Shi,[1] Yan Wang,[3] Jiaonan Wang,[1] Yingjian Zhang,[2] Xiaoyan Dong,[1] Ying Gao,[1] Yu Shen,[1] Li Dong,[1] Huichan Zhou,[1] Qinghua Sun,[1] Haoran Dong,[1] Xiumiao Peng,[2] Yi Zhang,[1] Meng Cao,[2] Hong Zhi,[2] Jingyang Zhou,[3] and Xiaoming Shi[1,*]

[1]China CDC Key Laboratory of Environment and Population Health, National Institute of Environmental Health, Chinese Center for Disease Control and Prevention, Beijing 100021, China

[2]Jinan Municipal Center for Disease Control and Prevention, Jinan, Shandong 250021, China

[3]Shandong Provincial Center for Disease Control and Prevention, Jinan, Shandong 250014, China

[4]These authors contributed equally

*Correspondence: shixm@chinacdc.cn

Received: August 7, 2021; Accepted: January 26, 2022; Published online: February 3, 2022; https://doi.org/10.1016/j.xinn.2022.100213

Citation: Li T., Fang J., Tang S., et al., (2022). $PM_{2.5}$ exposure associated with microbiota gut-brain axis: Multi-omics mechanistic implications from the BAPE study. The Innovation **3(2)**, 100213.

Recent studies have shown that $PM_{2.5}$ may activate the hypothalamus-pituitary-adrenal (HPA) axis by inducing hormonal changes, potentially explaining the increase in neurological and cardiovascular risks. In addition, an association between $PM_{2.5}$ and gut microbiota and metabolites was established. The above evidence represents crucial parts of the gut-brain axis (GBA). In view of this evidence, we proposed a hypothesis that $PM_{2.5}$ exposure may affect the HPA axis through the gastrointestinal tract microbiota pathway (GBA mechanism), leading to an increased risk of neurological and cardiovascular diseases. We conducted a real-world prospective repeated panel study in Jinan, China. At each visit, we measured real-time personal $PM_{2.5}$ and collected fecal and blood samples. A linear mixed-effects model was used to analyze the association between $PM_{2.5}$ and serum biomarkers, gut microbiota, and metabolites. We found that $PM_{2.5}$ was associated with increased serum levels of hormones, especially the adrenocorticotropic hormone (ACTH) and cortisol, which are reliable hormones of the HPA axis. Gut microbiota and tryptophan metabolites and inflammation, which are important components of the GBA, were significantly associated with $PM_{2.5}$. We also found links between $PM_{2.5}$ and changes in the nervous and cardiovascular outcomes, e.g., increases of 19.77% (95% CI: −36.44, 125.69) in anxiety, 1.19% (95% CI: 0.65, 1.74) in fasting blood glucose (FBG), 2.09% (95% CI: 1.48, 2.70) in total cholesterol (TCHOL), and 0.93% (95% CI: 0.14, 1.72) in triglycerides (TG), were associated with 10 $\mu g/m^3$ increase in $PM_{2.5}$ at the lag 0–72 h, which represent the main effects of GBA. This study indicated the link between $PM_{2.5}$ and the microbiota GBA for the first time, providing evidence of the potential mechanism for $PM_{2.5}$ with neurological and cardiovascular system dysfunction.

INTRODUCTION

The effects of fine particulate matter ($PM_{2.5}$) have become an important global public health concern. Ambient $PM_{2.5}$ pollution has contributed to more than 1.42 million deaths in China, accounting for 34.3% of the total disease burden worldwide.[1] Extensive scientific evidence has shown that exposure to $PM_{2.5}$ has adverse health effect associations with cardiopulmonary diseases.[2–6] However, the biological mechanisms responsible for the health effects of $PM_{2.5}$ have not yet been fully elucidated. Systemic inflammation, oxidative stress, and epigenetic modifications are the main potential mechanisms reported in previous studies.[7–9] In recent years, emerging epidemiological evidence of the relationship between $PM_{2.5}$ and nervous system disease has received extensive attention.[10–12] The most recent relevant epidemiological studies have suggested a new mechanism involving $PM_{2.5}$-induced hormone alterations that are consistent with hypothalamus-pituitary-adrenal (HPA) axis activation, potentially explaining the increase in neurological and cardiovascular risk.[13,14]

Given the established link between $PM_{2.5}$ and HPA-related stress hormones, the question of how $PM_{2.5}$ induces such a neuroendocrine response arises. Studies have explored that $PM_{2.5}$ is inhaled into the respiratory tract to activate sensory nerves and to transmit signals to central nervous system regions that can lead to the stimulation of the HPA stress axis.[15–17] Beyond the activation of sensory nerves, whether other tracts are involved in the mechanism linking $PM_{2.5}$ and the HPA axis is still not clear. Most $PM_{2.5}$ (approximately 95%) is inhaled from the air through the mouth and nose and then passes through the blood barrier of the lungs, and the remainder (approximately 5%) is absorbed via the gastrointestinal tract.[18] Emerging animal experimental studies have shown that $PM_{2.5}$ may affect the gut microbiome by the gastrointestinal tract.[19–22] Epidemiological studies also proved that $PM_{2.5}$ can change the composition of gut microbiota.[23,24] The results of $PM_{2.5}$ dosing in humans and the latest studies on the link between $PM_{2.5}$ and gut microbiota have suggested the possibility that $PM_{2.5}$ exposure may affect the HPA axis through the gastrointestinal tract microbiota pathway (gut-brain axis [GBA] mechanism).

Recent studies have started to apply omics approaches such as nontargeted metabolomics, gut microbiome, and transcriptome analyses to explore the mechanisms linking $PM_{2.5}$ and disease, providing powerful tools for comprehensively understanding the biological pathways linking $PM_{2.5}$ and health effects.[25–31] Here, we conducted a prospective panel study of biomarkers and air pollutant exposure in healthy, elderly Chinese individuals (the China BAPE study) through analyses of the gut microbiome, untargeted serum metabolome, etc., to assess the changes in functional indications, biomarkers, metabolomics, and gut microbiome. We attempt to provide evidence of linking between $PM_{2.5}$ exposure and the microbiota GBA related to the progression of neurological and cardiovascular diseases.

METHODS

Study design and participants

The China BAPE study was performed according to a repeated-measurement panel design in Jinan, the capital city of Shandong Province. It was conducted from September 10, 2018, to January 19, 2019. We recruited participants from the Dianliu community of Jinan, in the proximity of a fixed-site monitoring station (approximately 1.5 km), and there were no factories within at least 5 km.

In the study, 76 healthy, elderly participants were recruited and participated. The participant exclusion criteria included the following: (1) diagnosis with chronic or acute disease; (2) any use of antibiotics, hormones, anti-inflammatory agents, or other medications in the past month; and (3) any detectable individual-level risk factors. More details are provided in the supplemental information. All participants were scheduled to participate in 5 repeated visits at monthly intervals from September 2018 to January 2019. Participants were asked to complete a detailed questionnaire including basic family information, personal information, and time-activity patterns.

The study protocol was approved by the Ethical Review Committee of the National Institute of Environmental Health, Chinese Center for Disease Control and Prevention (no. 201816). All participants provided written informed consent at enrollment.

Air pollution exposure measurements

We used MicroPEM sensors (v3.2, RTI, Research Triangle Park, NC, USA) to measure real-time personal $PM_{2.5}$ exposure continuously for 3 days for each visit. Participants were required to wear the sampler at all times. Real-time $PM_{2.5}$ concentrations were recorded every 10 s. The MicroPEM sensor measured the average concentration of personal $PM_{2.5}$ over 1 min and then stopped sampling for 3 min. The hourly average concentration of personal $PM_{2.5}$ was calculated on the basis of ≥75% effective data within 1 h (otherwise not available [NA]). All participants maintained their normal activities during the 3-day sampling period before the health check. We examined exposure to $PM_{2.5}$ in multiple, separate lag periods before the day of the health examinations, including 0–6, 0–12, 0–24, 0–36, 0–48, and

Table 1. Characteristics of the study population, $PM_{2.5}$, temperature, and relative humidity (mean ± SD or number [%])

Variable	Total (N = 76)	Visit 1: 2018.09 (N = 65)	Visit 2: 2018.10 (N = 73)	Visit 3: 2018.11 (N = 70)	Visit 4: 2018.12 (N = 71)	Visit 5: 2019.01 (N = 71)
Age	64.5 (4.5)	64.4 (4.7)	64.9 (2.9)	64.9 (2.8)	64.9 (2.9)	65.0 (2.9)
BMI, kg/m^2	25.1 (2.5)	24.8 (2.5)	25.1 (2.5)	25.1 (2.3)	25.1 (2.3)	25.0 (2.4)
Height, cm	162.5 (7.9)	161.9 (7.7)	162.5 (7.8)	162.9 (8.0)	162.5 (8.0)	162.8 (7.9)
Weight, kg	66.3 (9.1)	65.2 (8.8)	66.4 (9.1)	66.8 (9.3)	66.5 (8.9)	66.5 (9.1)
Income, wan yuan	10.0 (6.7)	10.4 (6.9)	9.8 (6.7)	10.3 (6.8)	10.1 (6.6)	10.4 (6.7)
Cotinine, μg/L	0.6 (2.4)	0.4 (0.1)	0.6 (2.5)	0.6 (3.4)	1.3 (5.0)	2.3 (10.9)
Gender						
Male	37 (48.7)	29 (44.6)	35 (47.9)	35 (50.0)	34 (47.9)	37 (52.1)
Female	39 (51.3)	36 (55.4)	38 (52.1)	35 (50.0)	37 (52.1)	34 (47.9)
Education						
Below primary school	5 (6.6)	5 (7.7)	5 (6.9)	4 (5.7)	5 (7.0)	5 (7.0)
Primary school	3 (4.0)	2 (3.1)	3 (4.1)	2 (2.9)	2 (2.8)	1 (1.4)
Junior high school	21 (27.6)	17 (26.2)	21 (28.8)	19 (27.2)	19 (26.8)	20 (28.2)
Senior high school	33 (43.4)	29 (44.6)	32 (43.8)	31 (44.3)	31 (43.7)	31 (43.7)
University	14 (18.4)	15 (23.1)	12 (16.4)	14 (20.0)	14 (19.7)	14 (19.7)
Drink						
Yes	2 (2.6)	1 (1.5)	2 (2.7)	3 (4.3)	1 (1.4)	1 (1.4)
No	74 (97.4)	64 (98.5)	71 (97.3)	67 (95.7)	70 (98.6)	70 (98.6)
Cook						
Yes	65 (85.5)	54 (83.1)	62 (84.9)	61 (87.1)	64 (90.1)	63 (88.7)
No	11 (14.5)	11 (16.9)	11 (15.1)	9 (12.9)	7 (9.9)	8 (11.3)
Anxiety						
Yes	3 (3.9)	3 (4.6)	2 (2.7)	9 (12.9)	0 (0)	0 (0)
No	73 (96.1)	62 (95.4)	71 (97.3)	61 (87.1)	71 (100)	71 (100)
Sleep disorder						
Yes	14 (18.4)	14 (21.5)	9 (12.7)	9 (12.9)	15 (21.1)	14 (19.7)
No	62 (81.6)	51 (78.5)	64 (87.3)	61 (87.1)	56 (78.9)	57 (80.3)
$PM_{2.5}$, $\mu g/m^3$	57.1 (44.9)	54.03 (30.6)	32.4 (16.1)	57.9 (16.9)	51.0 (28.4)	90.7 (78.4)
Temperature, °C	21.7 (3.4)	26.3 (3.3)	20.9 (1.64)	21.6 (2.25)	20.1 (2.6)	19.9 (2.3)
Relative humidity, %	45.7 (12.9)	63.3 (13.1)	44.6 (7.45)	46.3 (8.02)	37.3 (7.3)	38.0 (8.5)

0–72 h. Personal temperature and relative humidity data were also recorded by the MicroPEM system.

Clinical and biomarker measurements

We collected fecal samples to evaluate distinct 16S rRNA gene regions, including 16S V4, 16S V3-V4, and 16S V4-V5. Phusion High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA) was used to carry out all PCR assays. We added index codes as recommended by the manufacturer and used the TruSeq DNA-PCR-Free Sample Preparation Kit (Illumina, San Diego, CA, USA) to generate sequencing libraries. We used the Illumina HiSeq 2500 platform to sequence the library, and 250 bp paired-end reads were generated. UPARSE software (UPARSE v.7.0.1001, http://drive5.com/uparse/) was used to perform sequence analysis, and sequences with ≥97% similarity were assigned to the same operational taxonomic units (OTUs). A standard sequence number corresponding to the sample with the fewest sequences was used to normalize the OTU abundance information.

We collected blood samples for untargeted metabolomic profiling studies. The automated Micro-Lab STAR system from the Hamilton Company (Reno, NV, USA) was used to prepare samples and ultrahigh-performance liquid chromatography-mass spectrometry (UPLC-MS) was used to analyze samples. Ion peaks from UPLC-MS were annotated with the Human Metabolome Database (HMDB), and the Discovery HD4 Metabolomics Platform and peaks were quantified using the area under the curve. The identification of metabolites in samples requires strict matching of three criteria between experimental data and library entry to ensure that the identification of all metabolites is highly reliable: (1) narrow window retention time, (2) accurate mass with variation less than 10 ppm, and (3) tandem MS (MS/MS) spectra with high forward and reverse searching scores. We performed a data normalization step to correct variation resulting from instrument tuning differences for studies spanning multiple days.

We used the Merck MILLIPLEX Human Cardiovascular Disease (Acute Phase) panel to measure serum biomarkers, including hormones and inflammatory, neural, and cardiovascular biomarkers. We performed all tests according to the manufacturer's instructions.

During the physical examination of all subjects, fasting venous blood samples were collected and routinely tested at Ankang Clinical Hospital. We used a Roche Cobas c702 system (Santa Clara, CA, USA) from Dian Diagnostics (Hangzhou, China) to measure blood biochemical indicators, including fasting blood glucose (FBG), total cholesterol (TCHOL), and triglycerides (TG).

Table 2. Descriptive statistics of biomarkers for the study participants (N = 350)

Variable	Mean (SD)	Variables	Mean (SD)
Hormones		Neurokines	
ACTH, pg/mL	8.3 (21.0)	ApoE4, pg/mL	172,025.4 (399,525.6)
AGRP, pg/mL	20.7 (43.4)	ferritin, pg/mL	344,590.1 (153,675.9)
C.Peptide, pg/mL	740.8 (335.7)	neurogranin, pg/mL	105.7 (125.3)
Cortisol, ng/mL	49 (16.3)	PRNP, pg/mL	29,767.4 (29,393.3)
GIP, pg/mL	36.9 (21.5)	cardiovascular biomarkers	
Leptin, pg/mL	4,018.7 (3,742.8)	AGP, ng/mL	1,456.2 (538.9)
T3, ng/mL	1.5 (1.0)	CRP, ng/mL	8.5 (17.6)
T4, ng/mL	45.4 (13)	Fetuin-A, ng/mL	203.5 (55.6)
TSH, uiu/mL	5.0 (4.3)	haptoglobin, ng/mL	1,112.8 (1,096.3)
Inflammations		SAP, ng/mL	7.6 (3.2)
IL.10, pg/mL	8.5 (46.2)	cardiovascular functional factors	
IL.23, pg/mL	307 (756.2)	FBG, mmol/L	6.5 (1.5)
IL.4, pg/mL	218.3 (165.5)	TCHOL, mmol/L	5.8 (1.3)
IL.13, pg/mL	3.9 (3.4)	TG, mmol/L	1.6 (0.5)
MIP.3.alpha, pg/mL	15.9 (55.5)		
TNF.alpha, pg/mL	3.7 (1.4)		

Functional scale questionnaire

We used the Generalized Anxiety Disorder scale (GAD7) and the Pittsburgh Sleep Quality Index (PSQI) to investigate whether participants had anxiety and/or sleep disorders. All interviewers received training and used electronic questionnaires to conduct face-to-face surveys with respondents in the hospital. Electronic questionnaires are cheap and convenient and make it easy to achieve quality control in data validation and logic verification, and data can be easily merged and exported. The GAD7 represents an anxiety measure based on seven items scored from zero to three. The whole-scale scores ≤ 4 represent no anxiety, and scores >4 indicate anxiety symptoms. The PSQI is composed of 19 questions, reflecting seven main components. An overall score of <5 indicates "good" sleep quality, and ≥ 5 indicates "bad" sleep quality.

Statistical analysis

We applied a linear mixed-effects (LME) model to estimate the association between $PM_{2.5}$ and biomarkers, the gut microbiota, and metabolomics data. A generalized linear mixed-effects model with logistic regression was used to estimate the association between $PM_{2.5}$ and anxiety and sleep disorders. Before statistical analysis, a logarithmic transformation was performed on the biomarkers that did not show a normal distribution. We included several covariates in the models: (1) age, sex, BMI, and annual income; (2) education status and cooking and drinking habits; (3) blood cotinine level; (4) day of the week; and (5) a natural-spline function of personal temperature and relative humidity, both with 3 degrees of freedom. The Benjamini-Hochberg false discovery rate (FDRB-H) method was used to account for multiple testing to adjust the probability of type I error (p) value.[32] If the test statistics (or p values) are independently distributed or the joint distribution of the test statistics is dependent on the positive regression distribution (PRDS) of each subset of the true null hypothesis or are in the family of all pairwise comparisons of means in a balanced one-way layout with normal errors, FDRB-H can control the FDR of level α.[33] It has been shown that FDRB-H has excellent power to detect real differences, where FDRB-H <0.05 was considered statistically significant. Sensitivity analysis was conducted to examine the robustness of our results to remove or replace these covariates (Table S1). We reported the effect estimates as a percentage change in biomarkers per increase in 10 $\mu g/m^3$ of the $PM_{2.5}$ concentration.

All analyses were performed using R v.3.6.1 (R Development Core Team, 2006) with the lme4 package.

RESULTS

Descriptive analysis

The descriptive statistics of the 76 participants and the average $PM_{2.5}$ concentrations measured for 3 days before health examinations conducted during five visits are summarized in Table 1. Although all subjects were nonsmokers, the average level of plasma cotinine, a reliable biomarker of smoking, was 0.6 (2.4) $\mu g/L$. The average personal $PM_{2.5}$ concentration measured during the 3 days before the health examinations was 57.1 $\mu g/m^3$. The descriptive statistics of the examined biomarkers are provided in Table 2. We also presented the average personal $PM_{2.5}$ concentrations, temperature, and humidity measured before the health examinations in different lag periods (0–6, 0–12, 0–24, 0–48, and 0–72 h) (Table S2). The biomarkers measured and behavioral risk factors recorded during the five visits are also presented (Table S3).

Gut microbiota and tryptophan metabolism

According to species annotation, we obtained the relative abundance of 516 species of the gut microbiota. A total of 20 significant gut microbiota, including both beneficial and harmful bacteria, were associated with $PM_{2.5}$ exposure (Figure 1). Untargeted metabolomics profiling revealed 601 metabolites with unique Human Metabolome Database IDs, and 253 metabolites showed a statistically significant (FDRB-H < 0.05) association with $PM_{2.5}$ exposure. Using MetaboAnalyst, we identified the characteristic GBA-tryptophan metabolic pathway. We found 4 significant tryptophan metabolites associated with $PM_{2.5}$ exposure. As shown in Figure 2, each 10 $\mu g/m^3$ increase in the $PM_{2.5}$ exposure level was associated with a 0.85% (95% CI: 0.14, 1.56) increase in 3-indoxyl sulfate, a 0.63% (95% CI: −0.15, 1.42) increase in anthranilate, a 0.27% (95% CI: −0.15, 0.70) increase in N-acetyltryptophan, and a −0.23% (95% CI: −0.42, −0.04) decrease in tryptophan in the 0–72 h lag.

Inflammation

We found that 6 inflammatory markers were significantly associated with 10 $\mu g/m^3$ increases in $PM_{2.5}$ (Figure 2). A 10 $\mu g/m^3$ increment in $PM_{2.5}$ exposure level at lag 0–72 h was associated with a −1.85% (95% CI: −3.57, −0.11) reduction in interleukin-4 (IL-4); a −2.18% (95% CI: −3.60, −0.75) reduction in IL-10; a −0.69% (95% CI: −3.06, −1.73) reduction in IL-13; and a −2.98% (95% CI: −4.97, −1.24) reduction in IL-23. The corresponding reduction in tumor necrosis factor alpha (TNF-α) was −1.94% (95% CI: −2.72, −1.15). Although macrophage inflammatory protein-3α (MIP3-α) and $PM_{2.5}$ were not significantly associated with $PM_{2.5}$, MIP3-α was positively correlated with $PM_{2.5}$. As shown in the sensitivity analyses, the overall observed associations remained robust (Table S4).

Hormones

We found 9 hormones that were associated with $PM_{2.5}$ exposure (Figure 2). Elevated levels of the adrenocorticotropic hormone (ACTH) were associated with each 10 $\mu g/m^3$ increase in $PM_{2.5}$ exposure, of which the most significant increase was 3.15% (95% CI: 1.56, 4.77). Significant increases in cortisol levels were also observed in association with exposure to $PM_{2.5}$. We have found positive associations of 10 $\mu g/m^3$ increase in $PM_{2.5}$ with a 0.69% (95% CI: 0.13, 1.24) increase in cortisol. Seven hormones (agouti gene-related protein [AGRP], C-peptide, gastric inhibitory peptide [GIP], leptin, thyroid-stimulating hormone [TSH], three iodine thyroids [T3], and thyroxin [T4]) related to the HPA axis were also associated with $PM_{2.5}$ exposure, although some presented no significant effect. As shown in the sensitivity analyses, the overall observed associations remained robust (Table S4).

Neurokines and nerve-related outcomes

Four neurokines were associated with $PM_{2.5}$ exposure. Each 10 $\mu g/m^3$ increase in $PM_{2.5}$ was associated with a 2.40% (95% CI: 0.62, 4.21) increase in apolipoprotein E (ApoE4) and a 0.75% (95% CI: −0.39, 1.91) increase in prion protein (PRNP). Significant decreases in ferritin (−1.40%, 95% CI: −2.41, −0.38) and neurogranin (−2.14%, 95% CI: −0.08, −4.16) were also observed in association with $PM_{2.5}$ (Figure 2). Results showed an increased risk of anxiety of 19.77% [95% CI: −36.44, 125.69] for each 10 $\mu g/m^3$ $PM_{2.5}$ increase at lag 0–72 h. The risk of sleep disorder increased significantly, by 4.82% (95% CI: −3.59, 13.96), for each 10 $\mu g/m^3$ $PM_{2.5}$ increase at lag 0–24 h (Figure 2). Sensitivity analyses showed that the overall associations observed remained robust (Table S4).

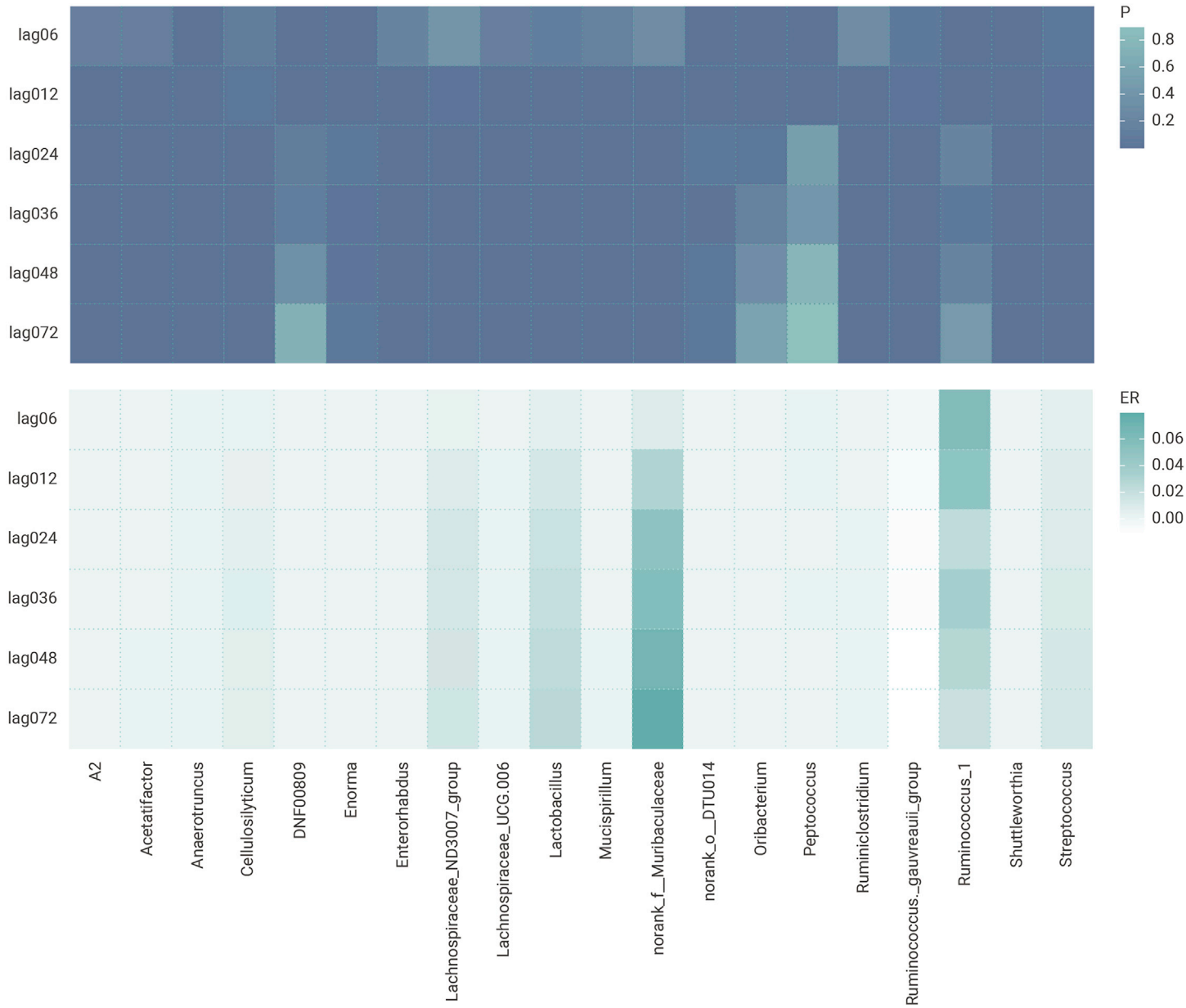


Figure 1. All gut microbiota components associated with $PM_{2.5}$ Cells are shaded according to the strength (i.e., p value and excess risk (ER)) of the association between each of the gut microbiota members associated with each single $PM_{2.5}$ lag.

Cardiovascular biomarkers and cardiovascular functional factors

Several cardiovascular biomarkers, including C-reactive protein (CRP), fetuin-A, α-acid glycoprotein (AGP), serum amyloid protein (SAP), and haptoglobin, were increased in association with $PM_{2.5}$ exposure. At the 0–72 h lag, each 10 μg/m^3 increase in the $PM_{2.5}$ exposure level was correlated with increases of 1.33% (95% CI: −1.90, 4.67) in CRP; 0.26% (95% CI: −0.48, 1.01) in fetuin-A; 0.20% (95% CI: −0.78, 1.20) in AGP; 0.018% (95% CI: −0.97, 1.02) in SAP; and 1.17% (95% CI: −1.33, 3.73) in haptoglobin. Similar increases were observed for cardiovascular functional factors. Increases of 1.19% (95% CI: 0.65, 1.74) in FBG, 2.09% (95% CI: 1.48, 2.70) in TCHOL, and 0.93% (95% CI: 0.14, 1.72) in TG were correlated with 10 μg/m^3 increase in $PM_{2.5}$ at the 0–72 h lag (Figure 2). Sensitivity analyses showed that the overall associations observed remained robust (Table S4).

DISCUSSION

To our knowledge, this is the first study to systematically explore the effect of $PM_{2.5}$ exposure on changes in functional indications, biomarkers, metabolomics, and microorganisms related to neurological and cardiovascular health effects and to simultaneously perform gut microbial sequencing and an integrated metaomics analysis of the untargeted metabolome to study the underlying mechanism linking $PM_{2.5}$ and GBA. In this real-world panel study, we found significant changes in the gut microbiota, tryptophan metabolism, inflammation, and hormone biomarkers that were consistent with GBA activation. We also found significant changes in neurokines, cardiovascular biomarkers, and functional factors related to $PM_{2.5}$ exposure. Based on these findings, we speculate that $PM_{2.5}$ exposure may activate GBA by affecting gut microbiota, tryptophan metabolism, inflammatory factors, and important hormones of the HPA axis, leading to neurological and cardiovascular system dysfunction (Figure 3).

Hormones increased with $PM_{2.5}$ exposure, which may imply the activation of the HPA axis. Previous studies showed that short-term exposure to $PM_{2.5}$ may induce HPA axis activation.[13,14,16,17,34] Inhalation of $PM_{2.5}$ will promote the release of corticotropin-releasing hormone (CRH) and ACTH from the hypothalamus and stimulate the synthesis and release of cortisol, a reliable hormone of the HPA stress axis.[35] A study with a randomized, double-blind, crossover design was the first-ever epidemiological study to use a metabolomics approach to show that $PM_{2.5}$ may induce metabolic alterations that are consistent with HPA axis activation.[13] The observed increases in cortisol, ACTH, and CRH were related to $PM_{2.5}$ exposure. We found that $PM_{2.5}$ exposure was associated with increased serum levels of ACTH and cortisol, which is consistent with the findings of the previous studies.[13,14,16,17] We also found significant changes in other hormones,

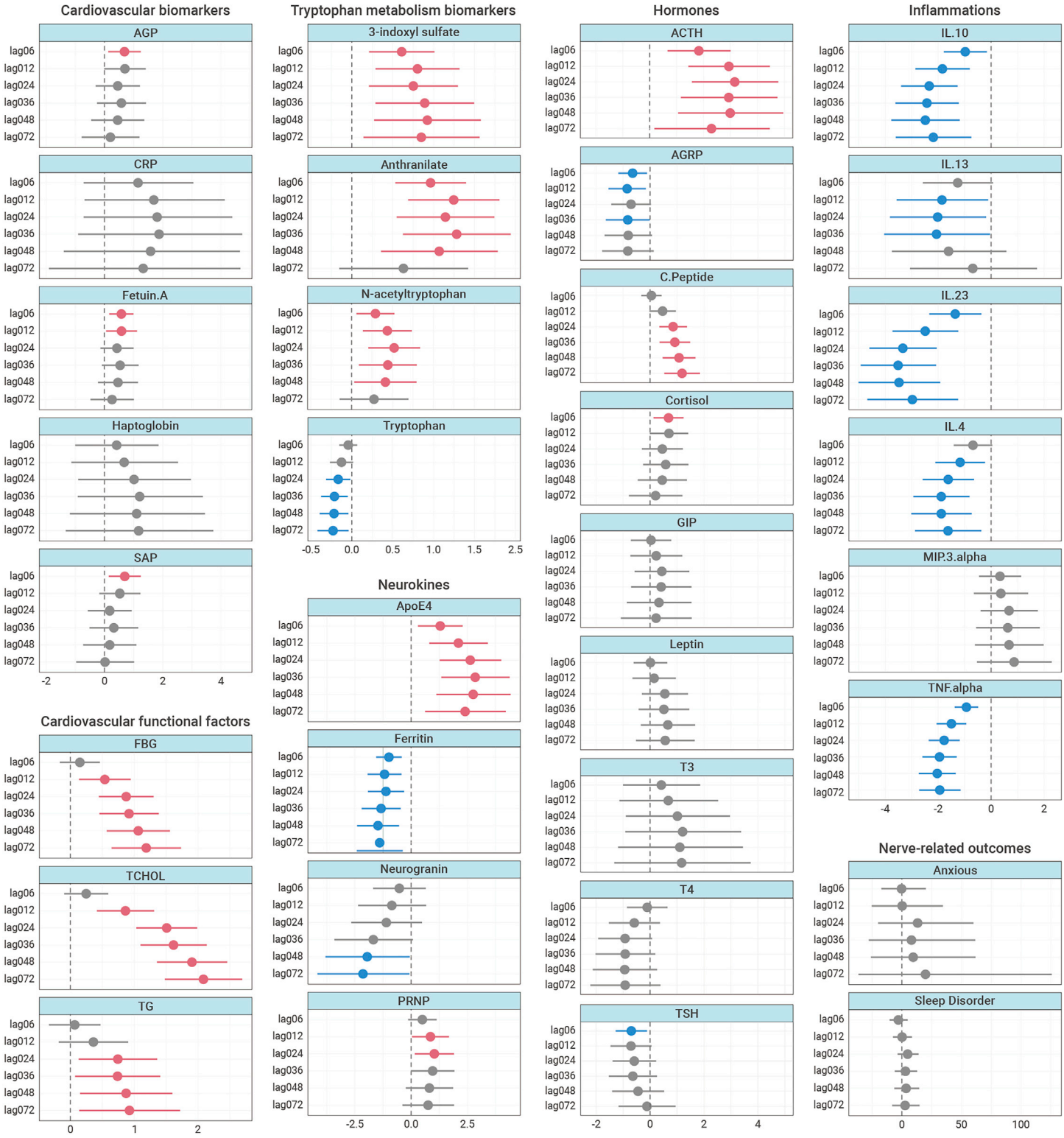


Figure 2. Percentage changes and 95% confidence intervals of biomarkers with each 10 μg/m^3 increase in $PM_{2.5}$ exposure.

especially AGRP and leptin, related to the HPA axis, which have had very few previous studies reported. An animal study showed that leptin reduced HPA axis activity to normal levels in diabetic mice.[36] Leptin acts via receptors on neurons in the hypothalamus. By inhibiting the expression of AGRP, it inhibits food intake and increases energy metabolism.[37,38] The significant changes in ACTH, cortisol, and other hormones related to $PM_{2.5}$ exposure observed in our study are presumably indicative of HPA stress axis activation.

We found significant changes in 20 members of the gut microbiota associated with $PM_{2.5}$ exposure, providing new insights into the relationship between $PM_{2.5}$ exposure and the gut microbiota. An animal experimental study demonstrated that $PM_{2.5}$ may affect the gut microbiota via the gastrointestinal tract; it showed that approximately 5% of the particulate matter taken in through the mouth and nose enters the gastrointestinal tract and significantly increases gut microbial diversity.[39] Liu et al. reported that the mice exposed to $PM_{2.5}$ exhibited increased proportions of the phyla Candidatus Saccharibacteria, Proteobacteria, and Fusobacteria and decreased proportions of the phyla Gemmatimonadetes, Acidobacteria, and Deferribacteres in the gut.[22] Li et al. also showed that ultrafine-particle-exposed mice presented an increase in Verrocomicrobia but a decrease in the

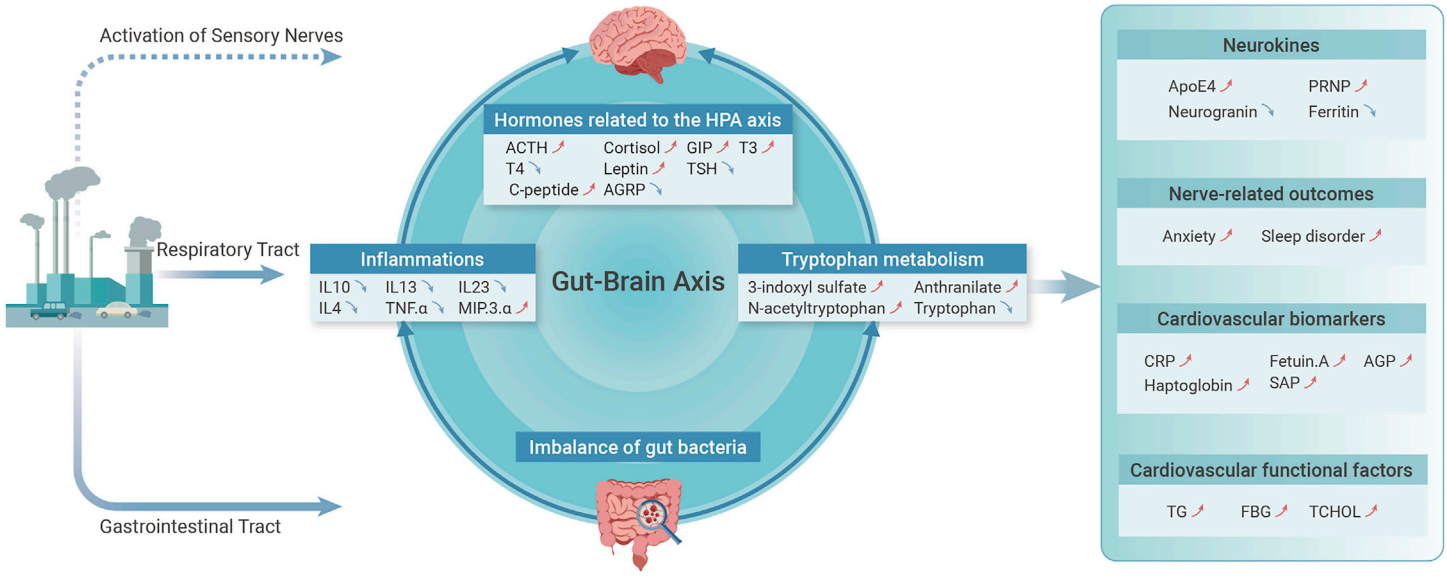


Figure 3. Summary of the GBA mechanism of this study Solid line represents the mechanism pathways discovered in this study, and dotted line represents the pathways that have been confirmed in previous studies.

phyla Cyanobacteria, Actinobacteria, and Firmicutes as well as reduced diversity in the microbiome.[21] In addition, an animal experimental study demonstrated that inhalation of diesel exhaust particles changed the composition of gut microbiota and resulted in colonic epithelial damage, accompanied by inflammatory cell infiltration and mucus consumption.[40] In contrast to the toxicologic studies on this topic, only two epidemiological studies have reported the correlations between gut microbiota and $PM_{2.5}$; these studies showed negative associations with all four α-diversity indices of the gut microbiota, while $PM_{2.5}$ is related to changes in the gut microbial phyla Firmicutes, Proteobacteria, Verrucomicrobia, and Bacteroidetes.[23,24]

Our study is the first to show that $PM_{2.5}$ exposure is significantly related to changes in tryptophan metabolism, which suggests potential GBA activation. Numerous published studies have proven that metabolomics can reflect internal metabolic disorders after exposure to $PM_{2.5}$.[5,41,42] Most studies have shown that $PM_{2.5}$ is related to changes in antioxidant pathways and metabolic products related to oxidative stress and inflammation. However, no previous study has reported a significant relationship between $PM_{2.5}$ and tryptophan metabolites. Epidemiological and toxicological studies have shown that tryptophan metabolism, which produces neuroactive metabolites, is an important component of GBA.[43–46] Studies have also shown that gut microbes may reduce the production of microbial metabolites (referred to as "neuroactive metabolites") through tryptophan metabolism, which in turn affects the function of the central and intestinal nervous systems.[47,48] Tryptophan can be converted to 5-hydroxytryptophan (5-HTP) by tryptophan hydroxylase, and 5-hydroxytryptamine (5-HT), the precursor of 5-HTP, is a key neurotransmitter involved in the regulation of mood at the level of the central nervous system (CNS).[49] Our study also provided evidence that $PM_{2.5}$ altered the gut microbiota composition and function. In summary, it can be speculated that $PM_{2.5}$ may cause an imbalance in the gut microbiome, resulting in changes in tryptophan metabolism and thereby activating GBA.

Our panel study suggested that short-term exposure to $PM_{2.5}$ resulted in significant changes in inflammatory factors in the blood, providing evidence of significant changes in both serum anti-inflammatory cytokines and pro-inflammatory cytokines. The limited epidemiological and toxicological studies conducted to date provide some evidence that systemic inflammation may play a role in $PM_{2.5}$-induced HPA axis activation, which is also an important characteristic of GBA.[50–52] The existing evidence indicates that inhalation of $PM_{2.5}$ may result in respiratory tract inflammation and oxidative stress.[53] Inflammatory biomarkers may enter the circulatory system and result in lung inflammation and systemic inflammation. Furthermore, the balance between the brain and the gut can be modulated by the immune system.[54] Mutlu et al. showed that gut exposure to $PM_{2.5}$ may contribute to increases in gut permeability through epithelial barrier disruption.[55] Increases in gut permeability have been linked with intestinal inflammation in several studies.[20,56] Therefore, a gut microbiota imbalance may also lead to systemic inflammation, which would affect the CNS and participate in the development of brain diseases. Based on the evidence of a relationship of $PM_{2.5}$ with gut microbiota found in this study, we may infer that the observed $PM_{2.5}$-related inflammation is a potential manifestation of the complex mechanisms resulting from the exposure of multiple tracts. These biological changes may support the hypothesis that $PM_{2.5}$ activates GBA via inflammation.

Significant changes in GBA mainly cause related changes in the nervous and cardiovascular systems.[50,57,58] Our study observed the association between $PM_{2.5}$ and neurokines, nerve-related outcomes, cardiovascular biomarkers, and cardiovascular functional factors, which are consistent with the main effects of GBA. There is not enough evidence to prove the effect of $PM_{2.5}$ on the level of neurokines, and the available evidence is mainly related to neurological diseases such as anxiety and depression.[11,50,59] In the present study, we found changes in anxiety and sleep disorders, which are consistent with previous studies.[60,61] We also found significant changes in ApoE4, PRNP, ferritin, and neurogranin; changes in these biomarkers may indicate the existence of neurological diseases.[62–64] Several studies have reported that $PM_{2.5}$ can increase neurological and cardiovascular risk. CRP, haptoglobin, SAP, fetuin-A, and AGP are markers of systemic inflammation and oxidation associated with cardiovascular diseases.[65–67] Moreover, previous studies have shown that FBG, TCHOL, and TG are associated with $PM_{2.5}$, which is consistent with the findings of our study.[67–69]

Our study has several strengths. First, it is an epidemiological study based on real-world, population-level studies exploring potentially relevant mechanisms. The examined $PM_{2.5}$ exposure range is very wide, reflecting the actual exposure of the study subjects themselves. Second, our study used a combination of metaomics and multi-indicator technology and explored the important hypothesis that $PM_{2.5}$ stimulates GBA via gut microbes, the HPA axis, tryptophan metabolism, and immune pathways, which was not found in previous studies. Third, the prospective repeated-measurement study design means that each participant can serve as their own control, providing better control over the potential interactions between individual exposure and other confounding factors. Fourth, we performed an accurate personal $PM_{2.5}$ exposure assessment, which may better reflect the actual exposure of the research subjects.

In addition, our study has some limitations. First, we conducted this study in a unique population of healthy older people, which may introduce selection bias. However, we applied a rigorous screening process and review procedures to ensure that the subjects met our study standards to the greatest degree possible. Second, nutrition and diet effects cannot be fully controlled, considering the long-term effects. Third, for untargeted metabolomics profiling, targeted metabolomics analysis could further confirm the screened tryptophan metabolites. In our untargeted metabolomics analysis, the metabolites were identified by

searching an in-house library generated from commercial standards instead of searching any online metabolite database, so we believe that our untargeted metabolomic analysis may be solid and reliable. Fourth, there may be five different communication routes between the gut microbiota and the brain. We explored only some of the pathways within the GBA axis due to technological limitations. In addition, our study is an observational, prospective, epidemiological study and provides only mechanistic implications for a link between $PM_{2.5}$ exposure and GBA in humans; further studies are needed to prove a causal relationship. Finally, we have only explored the relevant mechanisms of $PM_{2.5}$ mass exposure that activate the GBA axis, which leads to an increased risk of cardiovascular and neurological diseases, without considering the role of $PM_{2.5}$ components. In the future, using the strategy of exposome to further explore the mechanism between $PM_{2.5}$ components and cardiovascular and neurological diseases is highly recommended.[70]

CONCLUSIONS

Inhalation of $PM_{2.5}$ increased serum levels of hormones to the active HPA stress axis, which represent crucial parts of the GBA. $PM_{2.5}$ also altered the gut microbiota composition and function, and gut microbes may change tryptophan metabolism, thereby activating GBA. Short-term exposure to $PM_{2.5}$ resulted in significant changes in inflammatory factors in the blood, which may support the hypothesis that $PM_{2.5}$ activates GBA via inflammation. Links between $PM_{2.5}$ and changes in the nervous and cardiovascular outcomes represent the main effects of GBA. Therefore, our results support the hypothesis that $PM_{2.5}$ exposure may activate GBA by affecting gut microbiota, tryptophan metabolism, inflammatory factors, and important hormones of the HPA axis, leading to neurological and cardiovascular system dysfunction.

REFERENCES

1. GBD 2019 Risk Factors Collaborators (2020). Global burden of 87 risk factors in 204 countries and territories, 1990-2019: a systematic analysis for the global burden of disease study 2019. Lancet ***396***, 1223–1249.
2. Pope, C.A., Muhlestein, J.B., May, H.T., et al. (2006). Ischemic heart disease events triggered by short-term exposure to fine particulate air pollution. Circulation ***114***, 2443–2448.
3. Brook, R.D., Rajagopalan, S., Pope, C.A., et al. (2010). Particulate matter air pollution and cardiovascular disease. Circulation ***121***, 2331–2378.
4. Du, Y., Xu, X., Chu, M., et al. (2016). Air particulate matter and cardiovascular disease: the epidemiological, biomedical and clinical evidence. J. Thorac. Dis. ***8***, E8–E19.
5. Chen, C., Li, H., Niu, Y., et al. (2019). Impact of short-term exposure to fine particulate matter air pollution on urinary metabolome: a randomized, double-blind, crossover trial. Environ. Int. ***130***, 104878.
6. Hystad, P., Larkin, A., Rangarajan, S., et al. (2020). Associations of outdoor fine particulate air pollution and cardiovascular disease in 157436individuals from 21 high -income,middle -income,and low -incomecountries (PURE):a prospective cohort study. Lancet Planet. Health ***4***, e235–e245.
7. Li, W., Wilker, E.H., Dorans, K.S., et al. (2016). Short-term exposure to air pollution and biomarkers of oxidative stress: the framingham heart study. J. Am. Heart Assoc. ***5***, e002742.
8. Rich, D.Q., Kipen, H.M., Huang, W., et al. (2012). Association between changes in air pollution levels during the Beijing Olympics and biomarkers of inflammation and thrombosis in healthy young adults. JAMA ***307***, 2068–2078.
9. Rückerl, R., Hampel, R., Breitner, S., et al. (2014). Associations between ambient air pollution and blood markers of inflammation and coagulation/fibrinolysis in susceptible populations. Environ. Int. ***70***, 32–49.
10. Tallon, L.A., Manjourides, J., Pun, V.C., et al. (2017). Cognitive impacts of ambient air pollution in the national social health and aging project (NSHAP) cohort. Environ. Int. ***104***, 102–109.
11. Li, R.L., Ho, Y.C., Luo, C.W., et al. (2019). Influence of $PM_{2.5}$ exposure level on the association between Alzheimer's disease and allergic rhinitis: a national population-based cohort study. Int. J. Environ. Res. Publ. Health ***16***, 3357.
12. Pun, V.C., Manjourides, J., and Suh, H. (2017). Association of ambient air pollution with depressive and anxiety symptoms in older adults: results from the NSHAP study. Environ. Health Perspect. ***125***, 342–348.
13. Li, H., Cai, J., Chen, R., et al. (2017). Particulate matter exposure and stress hormone levels. Circulation ***136***, 618–627.
14. Niu, Y., Chen, R., Xia, Y., et al. (2018). Fine particulate matter constituents and stress hormones in the hypothalamus–pituitary–adrenal axis. Environ. Int. ***119***, 186–192.
15. Sirivelu, M.P., MohanKumar, S.M.J., Wagner, J.G., et al. (2006). Activation of the stress axis and neurochemical alterations in specific brain areas by concentrated ambient particle exposure with concomitant allergic airway disease. Environ. Health Perspect. ***114***, 870–874.
16. Balasubramanian, P., Sirivelu, M.P., Weiss, K.A., et al. (2013). Differential effects of inhalation exposure to $PM_{2.5}$ on hypothalamic monoamines and corticotrophin releasing hormone in lean and obese rats. Neurotoxicology ***36***, 106–111.
17. Chiarella, S.E., Soberanes, S., Urich, D., et al. (2014). β_2-adrenergic agonists augment air pollution-induced IL-6 release and thrombosis. J. Clin. Invest. ***124***, 2935–2946.
18. Sacks, J. (2009). Integrated Science Assessment for Particulate Matter (Second External Review Draft).
19. Wang, W., Zhou, J., Chen, M., et al. (2018). Exposure to concentrated ambient $PM_{2.5}$ alters the composition of gut microbiota in a murine model. Part Fibre. Toxicol. ***15***, 17.
20. Kish, L., Hotte, N., Kaplan, G.G., et al. (2013). Environmental particulate matter induces murine intestinal inflammatory responses and alters the gut microbiome. PLoS One ***8***, e62220.
21. Li, R., Yang, J., Saffari, A., et al. (2017). Ambient ultrafine particle ingestion alters gut microbiota in association with increased atherogenic lipid metabolites. Sci. Rep. ***7***, 42906.
22. Liu, W., Zhou, Y., Yong, li., et al. (2020). Effects of $PM_{2.5}$ exposure during gestation on maternal gut microbiota and pregnancy outcomes. Chemosphere ***247***, 125879.
23. Liu, T., Chen, X., Xu, Y., et al. (2019). Gut microbiota partially mediates the effects of fine particulate matter on type 2 diabetes: evidence from a population-based epidemiological study. Environ. Int. ***130***, 104882.
24. Bailey, M.J., Naik, N.N., Wild, L.E., et al. (2020). Exposure to air pollutants and the gut microbiota: a potential link between exposure, obesity, and type 2 diabetes. Gut Microb. ***11***, 1188–1202.
25. Fiehn, O. (2002). Metabolomics-the link between genotypes and phenotypes. Plant Mol. Biol. ***48***, 155–171.
26. Bonvallot, N., Tremblay-Franco, M., Chevrier, C., et al. (2014). Potential input from metabolomics for exploring and understanding the links between environment and health. J. Toxicol. Environ. Health B Crit. Rev. ***17***, 21–44.
27. Huang, Q., Hu, D., Wang, X., et al. (2018). The modification of indoor $PM_{2.5}$ exposure to chronic obstructive pulmonary disease in Chinese elderly people: a meet-in-metabolite analysis. Environ. Int. ***121***, 1243–1252.
28. Ayoung, J., Giovanni, F., Pekka, K.R., et al. (2018). Perturbation of metabolic pathways mediates the association of air pollutants with asthma and cardiovascular diseases. Environ. Int. ***119***, 334–345.
29. Ladva, C.N., Golan, R., Liang, D., et al. (2018). Particulate metal exposures induce plasma metabolome changes in a commuter panel study. PLoS One ***13***, e0203468.
30. Valles-Colomer, M., Falony, G., Darzi, Y., et al. (2019). The neuroactive potential of the human gut microbiota in quality of life and depression. Nat. Microbiol. ***4***, 623.
31. Heintz-Buschart, A., May, P., Laczny, C.C., et al. (2016). Integrated multi-omics of the human gut microbiome in a case study of familial type 1 diabetes. Nat. Microbiol. ***2***, 16227.
32. Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. Roy. Stat. Soc. B Stat. Methodol. ***57***, 289–300.
33. Kwong, K.S., Holland, B., and Cheung, S.H. (2002). A modified benjamini-hochberg multiple comparisons procedure for controlling the false discovery rate. J. Stat. Plan Infer. ***104***, 351–362.
34. Thomson, E.M. (2019). Air pollution, stress, and allostatic load: linking systemic and central nervous system impacts. J. Alzheimers Dis. ***69***, 597–614.
35. Spencer, R.L., and Deak, T. (2016). A users guide to HPA axis research. Physiol. Behav. ***178***, 43–65.
36. Clark, K.A., Jacob, E.B., Mohankumar, P.S., and Mohankumar, S. (2018). Leptin and HPA axis activity in diabetic rats: effects of adrenergic agonists. Brain Res. ***1707***, 54–61.
37. Warne, J.P., Varonin, J.M., Nielsen, S.S., et al. (2013). Coordinated regulation of hepatic energy stores by leptin and hypothalamic agouti-related protein. J. Neurosci. ***33***, 11972.
38. Goncalves, G.M., Li, W., Garcia, C.G., et al. (2014). Hypothalamic agouti-related peptide neurons and the central melanocortin system are crucial mediators of leptin's antidiabetic actions. Cell Rep. ***7***, 1093–1103.
39. Mutlu, E.A., Comba, I.Y., Cho, T., et al. (2018). Inhalational exposure to particulate matter air pollution alters the composition of microbiome in small bowel and colon. Environ. Pollut. ***240***, 817–830.
40. Li, X., Cui, J., Yang, H., et al. (2019). Colonic injuries induced by inhalational exposure to particulate-matter air pollution. Adv. Sci. ***6***, 1900180.
41. Mu, L., Niu, Z., Blair, R.H., et al. (2019). Metabolomics profiling before, during, and after the Beijing Olympics: a panel study of within-individual differences during periods of high and low air pollution. Environ. Health Perspect. ***127***, 57010.
42. Liang, D., Moutinho, J.L., Golan, R., et al. (2018). Use of high-resolution metabolomics for the identification of metabolic signals associated with traffic-related air pollution. Environ. Int. ***120***, 145–154.
43. Gheorghe, C.E., Martin, J.A., Manriquez, F.V., et al. (2019). Focus on the essentials: tryptophan metabolism and the microbiome-gut-brain axis. Curr. Opin. Pharmacol. ***48***, 137–145.
44. Gao, K., Mu, C.L., Aitak, F., and Zhu, W.Y. (2020). Tryptophan metabolism: a link between the gut microbiota and brain. Adv. Nutr. ***11***, 709–723.
45. Kaur, H., Bose, C., and Mande, S.S. (2019). Tryptophan metabolism by gut microbiome and gut-brain-axis: an in silico analysis. Front. Neurosci. ***13***, 1365.
46. Bosi, A., Banfi, D., Bistoletti, M., et al. (2020). Tryptophan metabolites along the microbiota-gut-brain axis: an interkingdom communication system influencing the gut in health and disease. Int. J. Tryptophan Res. ***13***, 1–25.
47. Desbonnet, L., Garrett, L., Clarke, G., et al. (2008). The probiotic bifidobacteria infantis: an assessment of potential antidepressant properties in the rat. J. Psychiatr. Res. ***43***, 164–174.
48. Kim, N., Yun, M., Oh, Y.J., et al. (2018). Mind-altering with the gut: modulation of the gut-brain axis with probiotics. J. Microbiol. ***56***, 172–182.
49. O'Mahony, S.M., Clarke, G., Borre, Y.E., et al. (2015). Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. Behav. Brain Res. ***277***, 32–48.
50. Zanobetti, A., Dominici, F., Wang, Y., and Schwartz, J.D. (2014). A national case-crossover analysis of the short-term effect of $PM_{2.5}$ on hospitalizations and mortality in subjects with diabetes and neurological disorders. Environ. Health ***13***, 38.

51. Tyler, C.R., Zychowski, K.E., Sanchez, B.N., et al. (2016). Surface area-dependence of gas-particle interactions influences pulmonary and neuroinflammatory outcomes. Part. Fibre Toxicol. ***13***, 64.
52. Chen, X., Gianferante, D., Hanlin, L., et al. (2017). HPA-axis and inflammatory reactivity to acute stress is related with basal HPA-axis activity. Psychoneuroendocrinology ***78***, 168–176.
53. Riggs, D.W., Zafar, N., Krishnasamy, S., et al. (2020). Exposure to airborne fine particulate matter is associated with impaired endothelial function and biomarkers of oxidative stress and inflammation. Environ. Res. ***180***, 108890.
54. Bengmark, S. (2013). Gut microbiota, immune development and function. Pharmacol. Res. ***69***, 87–113.
55. Mutlu, E.A., Engen, P.A., Soberanes, S., et al. (2011). Particulate matter air pollution causes oxidant-mediated increase in gut permeability in mice. Part Fibre. Toxicol. ***8***, 19.
56. Salim, S.Y., and Söderholm, J.D. (2011). Importance of disrupted intestinal barrier in inflammatory bowel diseases. Inflamm. Bowel Dis. ***17***, 362–381.
57. Yang, T., and Zubcevic, J. (2017). Gut-brain axis in regulation of blood pressure. Front. Physiol. ***8***, 845.
58. Quigley, E.M.M. (2017). Microbiota-brain-gut axis and neurodegenerative diseases. Curr. Neurol. Neurosci. ***17***, 94.
59. Choi, K.-H., Bae, S., Kim, S., and Kwon, H.-J. (2020). Indoor and outdoor $PM_{2.5}$ exposure, and anxiety among schoolchildren in Korea: a panel study. Environ. Sci. Pollut. Res. ***27***, 27984–27994.
60. Lawrence, W.R., Yang, M., Zhang, C., et al. (2018). Association between long-term exposure to air pollution and sleep disorder in Chinese children: the seven northeastern cities study. Sleep ***41***, 1–10.
61. Safieh, M., Korczyn, A.D., and Michaelson, D.M. (2019). ApoE4: an emerging therapeutic target for Alzheimer's disease. BMC Med. ***17***, 64.
62. Bagyinszky, E., Giau, V.V., Youn, Y.C., et al. (2018). Characterization of mutations in PRNP (prion) gene and their possible roles in neurodegenerative diseases. Neuropsychiatr. Dis. Treat. ***14***, 2067–2085.
63. Ayton, S., Faux, N.G., and Bush, A.I.; Alzheimer's Disease Neuroimaging Initiative. (2015). Ferritin levels in the cerebrospinal fluid predict Alzheimer's disease outcomes and are regulated by APOE. Nat. Commun. ***6***, 6760.
64. Portelius, E., Zetterberg, H., Skillbäck, T., et al. (2015). Cerebrospinal fluid neurogranin: relation to cognition and neurodegeneration in Alzheimer's disease. Brain ***138***, 3373–3385.
65. Zhang, J., Zhu, T., Kipen, H., et al. (2013). Cardiorespiratory biomarker responses in healthy young adults to drastic air quality changes surrounding the 2008 Beijing Olympics. Res. Rep. ***174***, 5–174.
66. Green, R., Broadwin, R., Malig, B., et al. (2016). Long- and short-term exposure to air pollution and inflammatory/hemostatic markers in midlife women. Epidemiology ***27***, 211–220.
67. Mathew, A.V., Yu, J., Guo, Y., et al. (2018). Effect of ambient fine particulate matter air pollution and colder outdoor temperatures on high-density lipoprotein function. Am. J. Cardiol. ***122***, 565–570.
68. Hwang, M.J., Kim, J.H., Koo, Y.S., et al. (2020). Impacts of ambient air pollution on glucose metabolism in Korean adults: a Korea national health and nutrition examination survey study. Environ. Health ***19***, 70.
69. Wu, X.M., Broadwin, R., Basu, R., et al. (2019). Associations between fine particulate matter and changes in lipids/lipoproteins among midlife women. Sci. Total Environ. ***654***, 1179–1186.
70. Fang, M., Hu, L., Chen, D., et al. (2021). Exposome in human health: utopia or wonderland. Innovation ***2***, 100172.

ACKNOWLEDGMENTS

This study was supported by the National Research Program for Key Issues in Air Pollution Control of China (no. DQGG0401), the National Natural Science Foundation of China (nos. 82025030 and 81941023), and the National Key Research and Development Program of China (no. 2016YFC0206500). We thank Calibra Lab at DIAN Diagnostics for the support with metabolomics analysis.

AUTHOR CONTRIBUTIONS

T.L. and X.S. designed the study. T.L. and H.D. performed data analysis and manuscript writing. H.D. performed data cleaning and preprocessing. J.F. and S.T. implemented the field study. All authors provided interpretation of the results and critical revisions.

DECLARATION OF INTERESTS

The authors declare no competing interests.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xinn.2022.100213.