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,2 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 Shi1,∗

*Correspondence: shixm@chinacdc.cn
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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
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 (TC), and 0.93% (95% CI: 0.14, 1.72) in triglycerides (TG), were associated with 10 µg/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. Extensive scientific evidence has shown that exposure to PM$_{2.5}$ has adverse health effects in various populations. 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. In recent years, emerging epidemiological evidence of the relationship between PM$_{2.5}$ and nervous system disease has received extensive attention. The most recent relevant epidemiological studies have suggested new mechanisms 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.

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. 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. Emerging animal experimental studies have shown that PM$_{2.5}$ may affect the gut microbiome via the gastrointestinal tract. Epidemiological studies also proved that PM$_{2.5}$ can change the composition of gut microbiota. 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. 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 of chronic or acute diseases; (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...
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 MILLPLEX 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 (TC/HOL), and triglycerides (TG).

| 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/m² | 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)                |
| PM2.5, μg/m³ | 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.
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 (355.7)| 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 | 1,456.2 (538.9) |
| Leptin, pg/mL     | 4,018.7 (3,742.8) | AGP, ng/mL | 8.5 (17.6) |
| T3, ng/mL         | 1.5 (1.0)  | CRP, ng/mL | 8.5 (17.6) |
| T4, ng/mL         | 45.4 (13)  | FetoLin-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 | 6.5 (1.5) |
| IL.23, pg/mL      | 307 (756.2) | FBG, mmol/L | 5.8 (1.3) |
| IL.4, pg/mL       | 218.3 (165.5)| TCHOL, mmol/L | 1.6 (0.5) |
| IL.13, pg/mL      | 3.9 (3.4)  | TG, mmol/L | 15.9 (55.5) |
| MIP.3.alpha, pg/mL| 3.7 (1.4)  | Neurokines | 2.41 (0.38) |
| TNF, alpha, pg/mL |           |            | 0.85 (1.56) |

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 viewers received training and used electronic questionnaires to conduct face-to-face surveys. 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 associations 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 associations 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-logarithmic transformation of each covariate. 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. The GAD7 was positively correlated with PM$_{2.5}$.

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) μg/mL. The average personal PM$_{2.5}$ concentration measured during the 3 days before the health examinations was 57.1 μg/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 BBA-tryptophan metabolic pathway. We found 4 significant tryptophan metabolites associated with PM$_{2.5}$ exposure. As shown in Figure 2, each 10 μg/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) change in tryptophan in the 0–72 h lag.

Inflammation

We found that 6 inflammatory markers were significantly associated with 10 μg/m$^3$ increases in PM$_{2.5}$ (Figure 2). A 10 μg/m$^3$ increase in PM$_{2.5}$ exposure level at lag 0–72 h was associated with a −1.85% (95% CI: −3.57, −0.11) change in interleukin-4 (IL-4); a −2.18% (95% CI: −3.60, −0.75) change in IL-10; a −0.69% (95% CI: −3.06, −1.73) change in IL-13; and a −2.98% (95% CI: −4.97, −1.24) change in IL-23. The corresponding change in tumor necrosis factor alpha (TNF-α) was −1.94% (95% CI: −2.72, −1.15). Although macrophage inflammatory protein-3α (MIP-3α) and PM$_{2.5}$ were not significantly associated with PM$_{2.5}$, MIP-3α 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 μg/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 association of 10 μg/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 μg/m$^3$ increase in PM$_{2.5}$ was associated with a 2.40% (95% CI: −3.57, −0.11) change in interleukin-4 (IL-4) and a 2.18% (95% CI: −3.60, −0.75) change in IL-10; a −0.69% (95% CI: −3.06, −1.73) change in IL-13; and a −2.98% (95% CI: −4.97, −1.24) change in IL-23. The corresponding change in tumor necrosis factor alpha (TNF-α) was −1.94% (95% CI: −2.72, −1.15). Although macrophage inflammatory protein-3α (MIP-3α) and PM$_{2.5}$ were not significantly associated with PM$_{2.5}$, MIP-3α was positively correlated with PM$_{2.5}$. As shown in the sensitivity analyses, the overall observed associations remained robust (Table S4).
Cardiovascular biomarkers and cardiovascular functional factors

Several cardiovascular biomarkers, including C-reactive protein (CRP), fetuin-A, a-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 $\mu$g/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 $\mu$g/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 multi-omics 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 neurokinins, 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. 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. 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. 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. We also found significant changes in other hormones,
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. Leptin acts via receptors on neurons in the hypothalamus. By inhibiting the expression of AGRP, it inhibits food intake and increases energy metabolism. The significant changes in ACTH, cortisol, and other hormones related to PM2.5 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 PM2.5 exposure, providing new insights into the relationship between PM2.5 exposure and the gut microbiota. An animal experimental study demonstrated that PM2.5 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. Liu et al. reported that the mice exposed to PM2.5 exhibited increased proportions of the phyla Candidatus Saccharibacteria, Proteobacteria, and Fusobacteria and decreased proportions of the phyla Gemmatimonadetes, Acidobacteria, and Deferribacteres in the gut. Li et al. also showed that ultrafine-particle-exposed mice presented an increase in Veerochromobacter but a decrease in the...
Numerous published studies have proven that metabolomics can re-
changes in tryptophan metabolism, which suggests potential GBA activation.
Epidemiological and toxicological studies have shown that tryptophan meta-
that PM2.5 altered the gut microbiota composition and function. In summary, it
gut exposure to PM2.5 may contribute to increases in gut permeability through
PM2.5 is related to changes in antioxidant pathways and metabolic products
Bacteroidetes.23,24
Our study is the first to show that PM2.5 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 PM2.5.47,48 Most studies have shown that
PM2.5 is related to changes in antioxidant pathways and metabolic products
related to oxidative stress and inflammation. However, no previous study has re-
ported a significant relationship between PM2.5 and tryptophan metabolites.
Epidemiological and toxicological studies have shown that tryptophan meta-
bolism, 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 tryp-
tophan 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 PM2.5 altered the gut microbiota composition and function. In summary, it
can be speculated that PM2.5 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 PM2.5 resulted in sig-
ificant 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 PM2.5-induced HPA axis activation, which is also an important char-
acteristic of GBA.50–52 The existing evidence indicates that inhalation of PM2.5
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 PM2.5 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 evi-
dence of a relationship of PM2.5 with gut microbiota found in this study, we
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These biological changes may support the hypothesis that PM2.5 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
PM2.5 and neurokines, nerve-related outcomes, cardiovascular biomarkers, and
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 PM2.5 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
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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 PM2.5 exposure and GBA in humans; further studies are needed to prove a causal relationship. Finally, we have only explored the relevant mechanisms of PM2.5 mass exposure that activate the GBA axis, which leads to an increased risk of cardiovascular and neurological diseases, without considering the role of PM2.5 components. In the future, using the strategy of exposure to further explore the mechanism between PM2.5 components and cardiovascular and neurological diseases is highly recommended.\(^\text{10}\)

**CONCLUSIONS**

Inhalation of PM2.5 increased serum levels of hormones to the active HPA stress axis, which represent crucial parts of the GBA. PM2.5 also altered the gut microbiota composition and function, and gut microbes may change tryptophan metabolism, thereby activating GBA. Short-term exposure to PM2.5 resulted in significant changes in inflammatory factors in the blood, which may support the hypothesis that PM2.5 activates GBA via inflammation. Links between PM2.5 and changes in the nervous and cardiovascular outcomes represent the main effects of GBA. Therefore, our results support the hypothesis that PM2.5 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.

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