Particulate Matter Exposure and Stress Hormone Levels

A Randomized, Double-Blind, Crossover Trial of Air Purification

Editorial, see p 628

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BACKGROUND: Exposure to ambient particulate matter (PM) is associated with a number of adverse health outcomes, but potential mechanisms are largely unknown. Metabolomics represents a powerful approach to study global metabolic changes in response to environmental exposures. We therefore conducted this study to investigate changes in serum metabolites in response to the reduction of PM exposure among healthy college students.

METHODS: We conducted a randomized, double-blind crossover trial in 55 healthy college students in Shanghai, China. Real and sham air purifiers were placed in participants' dormitories in random order for 9 days with a 12-day washout period. Serum metabolites were quantified by using gas chromatography-mass spectrometry and ultrahigh performance liquid chromatography-mass spectrometry. Between-treatment differences in metabolites were examined using orthogonal partial least square-discriminant analysis and mixed-effect models. Secondary outcomes include blood pressure, corticotropin-releasing hormone, adrenocorticotropic hormone, insulin resistance, and biomarkers of oxidative stress and inflammation.

RESULTS: The average personal exposure to PMs with aerodynamic diameters ≤2.5 μm was 24.3 μg/m^3 during the real purification and 53.1 μg/m^3 during the sham purification. Metabolomics analysis showed that higher exposure to PMs with aerodynamic diameters ≤2.5 μm led to significant increases in cortisol, cortisone, epinephrine, and norepinephrine. Between-treatment differences were also observed for glucose, amino acids, fatty acids, and lipids. We found significantly higher blood pressure, hormones, insulin resistance, and biomarkers of oxidative stress and inflammation among individuals exposed to higher PMs with aerodynamic diameters ≤2.5 μm.

CONCLUSIONS: This study suggests that higher PM may induce metabolic alterations that are consistent with activations of the hypothalamus-pituitary-adrenal and sympathetic-adrenal-medullary axes, adding potential mechanistic insights into the adverse health outcomes associated with PM. Furthermore, our study demonstrated short-term reductions in stress hormone following indoor air purification.

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Sources of Funding, see page 626

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Clinical Perspective

What Is New?

- This is the first-ever study to use the metabolomics approach with a randomized, double-blind, crossover design to explore biological mechanisms underlying the adverse health effects of particulate matter (PM) exposure.
- Higher PM exposure led to a significant increase in serum levels of stress hormones, suggesting activations of the hypothalamus-pituitary-adrenal and sympathetic-adrenal-medullary axes;
- We also observed metabolic changes in glucose, amino acids, and lipids in response to PM exposure.

What Are the Clinical Implications?

- Activations of the hypothalamus-pituitary-adrenal and sympathetic-adrenal-medullary axes may contribute to the adverse cardiovascular and metabolic effects of PM exposure.
- In China, indoor air purification is a practical way to reduce personal exposure to PMs and may improve cardiovascular health in the long run.

Convincing epidemiological evidence suggests that exposure to higher levels of ambient particulate matters (PMs), especially fine PMs with aerodynamic diameters ≤2.5 μm ($PM_{2.5}$), may have adverse cardiovascular and metabolic consequences such as hypertension, coronary heart disease, stroke, and diabetes mellitus.[1–4] Although potential biological mechanisms for these adverse health effects are yet to be fully ascertained, inflammation and oxidative stress are likely to be involved.[5–7] In addition, several studies have reported an association between PM exposure and dysfunction of the automatic nervous system, which is known to increase cardiovascular risk.[8–10] Animal studies further implicated $PM_{2.5}$ as a stressor to the central nervous system that might induce a cascade of neuroendocrine responses.[11,12] These provocative findings warrant mechanistic investigations in humans.

In the past decade, metabolomics has emerged as a powerful tool to understand metabolic changes, particularly of small molecules (<1000 Da), in response to pathophysiological conditions or environmental exposures.[13] With high-throughput technologies such as chromatography coupled with mass spectrometry or nuclear magnetic resonance spectroscopy, metabolomic analysis can efficiently separate and quantify a large number of compounds from a single biological sample, and thus identify novel molecules and pathways that are affected by diseases or environmental exposures. In recent years, metabolomics has been used to investigate global metabolic disruptions related to exposures of heavy metals, organic chemical compounds, and ozone.[14] However, to the best of our knowledge, few studies have used this approach to understand the global metabolic changes in response to PM.

Using the metabolomics approach, we conducted a randomized, double-blind, crossover trial of air purification to gain mechanistic insights into the adverse cardiovascular and metabolic changes associated with PM exposure. In addition to metabolomics, we also examined blood pressure and biomarkers of oxidative stress and inflammation as secondary outcomes of interest.

METHODS

Study Design and Participants

This randomized, double-blind, crossover trial was conducted from November to December 2015. We recruited 60 college students from 17 nonsmoking dormitories on Jiangwan Campus, Fudan University in Shanghai, China. All participants were healthy young adults with no history of allergic, respiratory, or cardiovascular diseases. To minimize potential indoor source of PM, we asked participants to refrain from cooking or housecleaning during the study period.

The 17 dormitories received alternate treatments in random order at each study period intermitted by a 12-day washout period. For the treatment of real purification, a high-efficiency air purifier was put in the middle of the dormitory, whereas, for the treatment of sham purification, we simply removed the filter gauze. According to the manufacturer, these air purifiers (model KJEA200E, 3M Filtrete, Shanghai, China) have a recommended cleaning area of 20 m^2 and a clean air delivery rate of 200 m^3/h. All treatments started at 7 PM on Friday of the week and ended at 7 AM the following Sunday. During each study period, all windows and doors were tightly closed. Other than taking classes, participants were required to stay in their dormitories as much as possible. A self-administered questionnaire of time-location was completed by all participants. At the end of each study period, we conducted a health test and collected samples within an hour.

The study protocol was registered at ClinicalTrials.gov (NCT02712333) and was approved by the Review Board of School of Public Health, Fudan University. All participants provided written informed consent at enrollment.

PM Exposure Measurements

We chose $PM_{2.5}$ as the main exposure of interest because of its well-documented adverse cardiovascular and metabolic outcomes.[4,15–18] Indoor $PM_{2.5}$ concentration in each dormitory was measured by using the MicroPEM Personal Exposure Monitor (RTI International) which was placed at least 1 m away from the purifier and ≈1.5 m above the ground. Real-time $PM_{2.5}$ concentrations were recorded every 10 seconds. This device does not have a display screen so participants did not know their exposure levels. In addition, we installed an outdoor $PM_{2.5}$ monitor (GRIMM EDM180, GRIMM Aerosol Technik GmBH&Co. KG) on the rooftop of the main building in Jiangwan Campus to measure outdoor $PM_{2.5}$ levels during the study. Last, we obtained hourly $PM_{2.5}$ data from 13 fixed-site monitoring stations across Shanghai. For each participant, we then calculated individual exposure to $PM_{2.5}$ during each

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study period by mapping the time and location data to the appropriate $PM_{2.5}$-monitoring data. To ensure the quality of monitoring data, we calibrated all monitoring devices at the beginning of each period. Temperature and relative humidity were recorded using temperature/relative humidity data loggers (HOBO UX100-003, Onset Computer Corporation), or collected from Shanghai Meteorologic Bureau.

Health Measurements and Sample Collection

Demographic information such as age, sex, height, weight, and disease history was collected at enrollment. Blood pressure was evaluated before the study and following each period. For each participant, left upper arm blood pressure was measured 3 times with measurement intervals of at least 2 minutes by a trained staff using a mercury sphygmomanometer. We used the second and third measurements to calculate mean systolic blood pressure (SBP) and diastolic blood pressure (DBP). Pulse pressure was calculated as the difference between mean SBP and DBP.

We collected peripheral blood and first-void morning urine from each participant at the end of each study period. Venous blood (3 mL) was collected after 12 hours of fasting by using vacuum collection tubes, water bathed at 37°C for 15 minutes, and then centrifuged at 3000 rpm for 10 minutes to extract serum. Morning urine (10 mL) was collected by using sterilized tubes. All samples were transported at 4°C within 2 hours and stored at –80°C until analysis. We conducted all examinations and collected samples at 7 AM on Sundays to minimize potential influence from physiological changes attributable to circadian rhythm.

Serum Metabolomics Analysis

Global metabolomics analysis was conducted by using gas chromatography-mass spectrometry (GC-MS) and ultrahigh performance liquid chromatography-mass spectrometry (UPLC-MS). GC-MS analysis was performed by using an Agilent 7890A gas chromatography incorporated with Agilent 5975C Time-of-Flight mass spectrometer platform (Agilent Technologies). UPLC-MS analysis was conducted with Agilent 1290 Infinity UPLC and Agilent 6538 Ultra High Definition and Accurate-Mass Quadrupole Time-of-Flight mass spectrometer platform (Agilent Technologies). Quality control and blank control samples were added into the sequence to assess the data repeatability. Detailed sample preparation, GC-MS and UPLC-MS acquisition, and quality control are described Methods in the online-only Data Supplement. Ion peaks generated from GC-MS were annotated in the NIST 11 standard mass spectral database and Fiehn database,[19] whereas ion peaks from UPLC-MS were annotated by searching the human metabolite database and the METLIN database.[20,21] All data were log-transformed for normalization and Pareto-scaled before statistical analysis.

Biomarker Measurements

We measured 4 biomarkers of oxidative stress, including urinary 8-hydroxy-2-deoxyguansine (with creatinine calibration), serum malondialdehyde, iso-prostaglandin F2α, and superoxide dismutase. These biomarkers reflect DNA oxidative damage (8-hydroxy-2-deoxyguansine), lipid peroxidation (malondialdehyde and iso-prostaglandin F2α), or antioxidant potential (superoxide dismutase), which have been examined extensively in previous studies on $PM_{2.5}$.[5,22–26] In addition, we measured 6 serum biomarkers of systemic inflammation (soluble CD40 ligand, high-sensitivity C-reactive protein, interleukin-1β, interleukin-6, tumor necrosis factor-α, and intercellular adhesion molecule-1) and 3 hormones (insulin, corticotropin-releasing hormone [CRH], and adrenocorticotropic hormone [ACTH]). We then calculated homeostatic model assessment of insulin resistance as an indicator of insulin resistance using the following formula: homeostatic model assessment of insulin resistance=fasting glucose (mg/dL)×insulin (mU/L)/405. Concentrations of 8-hydroxy-2-deoxyguansine, iso-prostaglandin F2α, CRH, ACTH, insulin, and all inflammatory biomarkers were measured with enzyme-linked immunosorbent assays, and glucose, malondialdehyde, and superoxide dismutase were measured with oxidase peroxidase, thiobarbituric acid–reactive substances, and water-soluble tetrazolium salt assays, respectively. All laboratory analyses were conducted according to the manufacturer's instructions.

Statistical Analysis

We have provided details of GC/UPLC-MS raw data preprocessing Methods in the online-only Data Supplement. In brief, raw data were processed by using the ChromaTOF software (GC-MS) (v4.24, LECO), with Agilent MassHunter Quantitative (UPLC-MS) and XCMS (UPLC-MS) for peak alignment, integration, and normalization to obtain data matrixes containing retention time, mass-to-charge ratio (*m/z*), peak intensity, and sample information. We then analyzed preprocessed data using a combined strategy of multivariate and univariate methods. First, we conducted unsupervised principal component analysis to examine the clustering of quality control samples. Second, we performed a supervised orthogonal partial least square-discriminant analysis (OPLS-DA) to discriminate serum metabolomes between the 2 treatments. Validation for OPLS-DA models was conducted in a 7-fold cross-validation process, and model overfitting was examined in a 999-time permutation test. We used R^2Y and Q^2 to evaluate the goodness-of-fit and predictive ability of each model,[27] and we used the variance importance in the projection (VIP) scores to define contributions from individual metabolites to the overall between-group difference in the OPLS-DA model.

For each metabolite with a VIP score >1.0, we used linear mixed-effect models to identify statistically significant differences between treatments with a fixed-effect binary variable for purification status (0 for sham and 1 for real purification) adjusting for age, sex, body mass index, and a random intercept for each participant. Results were adjusted for multiple comparisons using false discovery rate.[28] We then constructed mixed-effect models to examine the associations between 9-day average $PM_{2.5}$ exposure for each participant and log-transformed relative peak intensity for each metabolite, adjusting for age, sex, body mass index, a randomized intercept for each participant, and the mean temperature and relative humidity during study periods. All continuous covariates were mean centered before analysis. To identify metabolites that contributed the most to the between-treatment metabolome difference and were significantly associated with $PM_{2.5}$ exposure, we selected all metabolites with

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the following criteria: (1) a VIP score >1.0 from the OPLS-DA model; (2) *P* value for between-treatment difference <0.05 and false discovery rate <0.05; and (3) significant association with $PM_{2.5}$ exposure from linear mixed-effect models with false discovery rate <0.05. For each metabolite that was identified, we calculated fold changes between the sham purification (higher $PM_{2.5}$ exposure) and the real purification (lower $PM_{2.5}$ exposure), and percentage changes per 10 $\mu g/m^3$ increase of $PM_{2.5}$ exposure.

We used similar mixed-effect models to examine changes of blood pressure, urine, and serum biomarkers between treatments and in relation to $PM_{2.5}$ exposure. Last, we conducted 3 sensitivity analyses for all the health outcomes: (1) adding a random intercept for each dormitory in the model to control for possible dormitory effect, (2) including an indicator variable as a fixed-effect term in the model to examine potential period effects, and (3) including an interaction term (sex×$PM_{2.5}$) in the model to explore potential interactions with sex. For health outcomes that showed statistical interactions with sex, we further conducted stratified analyses.

Both principal component analysis and OPLS-DA were performed with SIMCA-P 14.0 (Umetrics). Analyses in linear mixed-effect models were conducted using the lme4 and lmerTest package of the R software (R Foundation for Statistical Computing). All statistical tests were 2-sided with α=0.05. Results were presented as percentage changes in health outcomes comparing the sham purification with the real purification, or as percentage changes for each 10 $\mu g/m^3$ increase of $PM_{2.5}$ exposure.

RESULTS

Baseline Description

A total of 60 participants with an equal number of males and females were recruited, and 55 (28 males and 27 females) completed the study (Figure 1). Baseline demographic characteristics and blood pressure measurements are summarized in Table 1. No participant left urban Shanghai during the study.

$PM_{2.5}$ Exposure

Table 2 presents the averages of outdoor and indoor $PM_{2.5}$ concentrations during the study. In both study periods, participants reported ≈75% of their time in their dormitories. As expected, the indoor $PM_{2.5}$ level was lower during real purification, on average 82% than that during sham purification. Furthermore, the averaged 24-hour personal exposure to $PM_{2.5}$ was also 54% lower during the real purification, at a level below the World Health Organization air quality guideline (24-hour average: 25 $\mu g/m^3$).

Serum Metabolites Identification

A total of 2213 peaks were detected by GC-MS and UPLC-MS from all 110 serum samples. Pooled quality control samples clustered tightly in principal component analysis models, indicating an excellent repeatability (Figure I in the online-only Data Supplement). Scoring plots generated from OPLS-DA models revealed a clear separation between the sham and real purification (Figure II in the online-only Data Supplement). Sevenfold cross-validations R^2Y and Q^2 indicated good fitness and predictability, and the negative Q^2 from 999-time permutation tests suggested no overfitting in OPLS-DA models (Table I in the online-only Data Supplement).

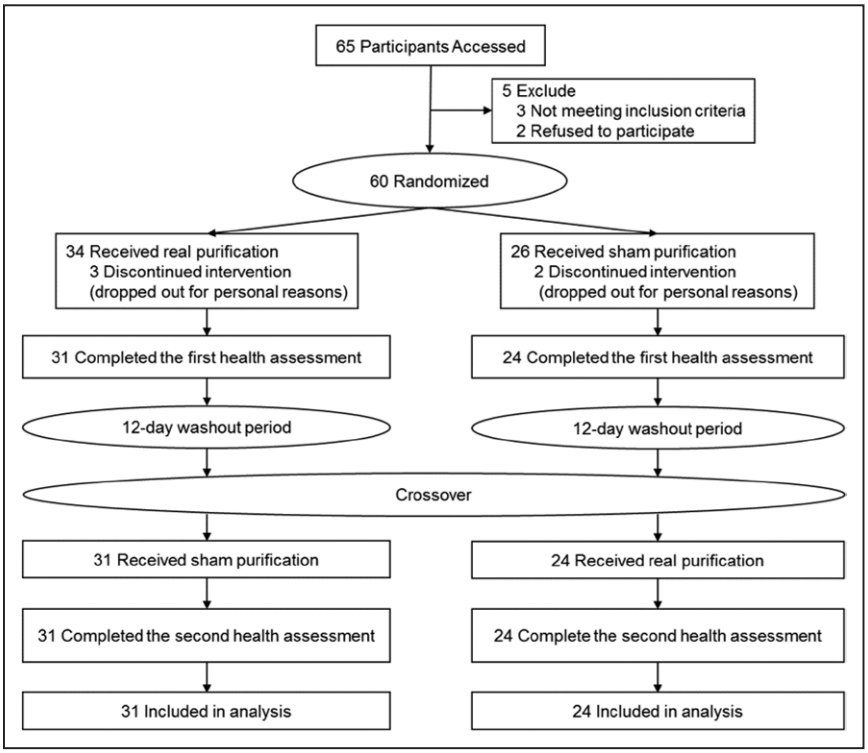

Figure 1. Flowchart of participation in a randomized, double-blind and crossover trial of air purification.

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Table 1. Characteristics of the Study Participants

	Mean (SD)
Age, y	20.2 (1.3)
Sex	
Male	28
Female	27
Body mass index, kg/m^2	21.1 (2.6)
Blood pressure, mm Hg	
Systolic pressure	106.4 (9.7)
Diastolic pressure	67.8 (6.5)
Pulse pressure	38.6 (7.3)

Last, 97 metabolites, including stress hormones, glucose, amino acids, fatty acids, lipids and their metabolites, were identified. Sensitivity analyses showed that our results were robust after further adjustment for dormitories and the fixed-effect term of period was not significant, suggesting no period effects (data not shown). Our exploratory interaction analyses showed significant interactions of $PM_{2.5}$ with sex for 5 metabolites (Figure III in the online-only Data Supplement).

Serum Stress Hormones in Relation to $PM_{2.5}$ Exposure

Of the 97 metabolites, we found higher levels of serum glucocorticoids (cortisone and cortisol), catecholamine (epinephrine and norepinephrine), and melatonin in association with higher $PM_{2.5}$ (Table 3). For example, serum cortisol and cortisone levels were 1.33 and 1.18 times higher during the sham over the real purification. Each 10 μg/m^3 increase in $PM_{2.5}$ exposure level was associated with 7.79% (95% confidence interval [CI], 4.75–10.91) increase in cortisol and 3.76% (95% CI, 1.84–5.71) increase in cortisone. The corresponding increases were 1.20-fold and 5.37% (95% CI, 3.30–7.48) for epinephrine and 1.57-fold and 11.70% (95% CI, 7.36–16.22) for norepinephrine. Similar increases were also observed for phenylalanine, tyrosine, and tetrahydropteridine, which are key amino acid precursors and enzyme cofactor in the catecholamine synthesis, and for the neurotransmitter melatonin and its precursor tryptophan, serotonin, and *N*-acetylserotonin (Figure 2).

Table 2. Indoor and Outdoor Daily Averages of $PM_{2.5}$ Concentrations, Temperature, and Relative Humidity During the Study

	Outdoor	Indoor	
		Sham Purification	Real Purification
$PM_{2.5}$, μg/m^3	101.4 (44.5)	46.8 (28.6)	8.6 (4.0)
Time-weighted average personal $PM_{2.5}$ exposure, μg/m^3*	–	53.1 (9.4)	24.3 (5.3)
Temperature, °C	12.4 (4.5)	19.9 (1.7)	20.2 (2.1)
Relative humidity, %	74.7 (18.7)	64.3 (7.4)	64.6 (7.8)

Values shown are mean (SD). $PM_{2.5}$ indicates particulate matter with aerodynamic diameter ≤2.5 μm.

*Time-weighted average personal $PM_{2.5}$ exposure was calculated by mapping the time and location data to the appropriate indoor or outdoor $PM_{2.5}$-monitoring data for each participant in each period.

Glucose, Amino Acid, and Lipid Metabolisms in Relation to $PM_{2.5}$ Exposure

$PM_{2.5}$ exposure was associated with serum levels of metabolites that are involved in metabolisms of glucose, amino acid, and lipid. As shown in Table II in the online-only Data Supplement, the serum levels of glucose and glucose 6-phosphate were significantly higher during sham purification and were associated with $PM_{2.5}$ exposure. Associations of serum metabolites with $PM_{2.5}$ exposure were also evident for 10 amino acids (arginine, leucine, histamine, threonine, serine, glutamine, lysine, phenylalanine, tyrosine, and tryptophan) and for derivatives or intermediate products of amino acid metabolism, as well (Table II in the online-only Data Supplement). Similar observations were made for free fatty acids and metabolites that are involved in fatty acid oxidation, including carnitine, acylcarnitine, hydroxy fatty acids, and acetone (Table III in the online-only Data Supplement). In addition, membrane phospholipids and their hydrolyzed products, including lysolipids and 2 eicosanoids, increased with higher $PM_{2.5}$ exposure (Table IV in the online-only Data Supplement). Last, 12 other metabolites, including purines and pyrimidines, were also found associated with $PM_{2.5}$ exposure (Table V in the online-only Data Supplement).

Blood Pressure and Biomarkers in Relation to $PM_{2.5}$ Exposure

We found higher blood pressures in association with higher $PM_{2.5}$ exposure (Figure 3). In comparison with the real purification, the SBP was 2.61% (95% CI, 0.39–4.79) higher during the sham purification, and each 10 μg/m^3 increase in $PM_{2.5}$ exposure was associated with 0.86% (95% CI, 0.10–1.62) increase in SBP; similar changes, albeit not statistically significant, were observed for DBP and pulse pressure. Using blood pressure at enrollment as the reference, we found significant decreases in both SBP and DBP during the real purification, but not during the sham purification (Figure IV in the online-only Data Supplement). The serum level of CRH was 28.03% (95% CI, 1.82–61.00) and of ACTH was 6.71% (95% CI, 0.51–12.53) higher in the sham purification than the real purification (Figure 4). When $PM_{2.5}$ was analyzed as a continuous variable

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Table 3. Changes in Glucocorticoids, Catecholamine, and Melatonin Synthesis Between Sham and Real Purification or With a per 10 μg/m³ Increase of $PM_{2.5}$ Exposure

Compound Name	VIP	Differences Between Treatments			Percentage Changes (95% CI) per 10 μg/m³ Increase of $PM_{2.5}$
		P Value	q Value	FC*	
Glucocorticoids					
Cortisol	1.25	9.87×10^{-7}	2.87×10^{-6}	1.33	7.79 (4.75 to 10.91)
Cortisone	1.27	7.57×10^{-6}	1.54×10^{-5}	1.18	3.76 (1.84 to 5.71)
Catecholamine synthesis					
Epinephrine	3.44	6.16×10^{-6}	1.29×10^{-5}	1.20	5.37 (3.30 to 7.48)
Norepinephrine	1.54	2.72×10^{-5}	4.43×10^{-5}	1.57	11.70 (7.36 to 16.22)
Phenylalanine	3.97	5.46×10^{-6}	1.18×10^{-5}	1.23	5.83 (3.51 to 8.20)
Tyrosine	2.25	4.23×10^{-7}	1.51×10^{-6}	1.25	5.99 (3.73 to 8.31)
Tetrahydropteridine	1.17	3.69×10^{-7}	1.36×10^{-6}	1.45	9.61 (6.27 to 13.05)
Melatonin synthesis					
L-Tryptophan	3.14	1.64×10^{-4}	2.12×10^{-4}	1.24	5.22 (2.52 to 7.99)
Serotonin	1.58	2.22×10^{-16}	4.84×10^{-15}	0.84	–5.03 (–6.21 to –3.84)
N-Acetylserotonin	2.04	2.32×10^{-7}	9.54×10^{-7}	1.56	12.88 (8.86 to 17.04)
Melatonin	2.91	2.53×10^{-6}	6.13×10^{-6}	1.34	8.52 (5.52 to 11.61)

CI indicates confidence interval; FC, fold change; $PM_{2.5,}$ particulate matters with aerodynamic diameters ≤2.5 μm; and VIP, variance importance in the projection scores.

*Fold change was calculated by sham/real purification.

in the mixed-effect model, however, the association was only statistically significant for CRH with a 6.96% (95% CI, 0.01–13.96) increase per 10 μg/m³ increase in $PM_{2.5}$ concentration. Furthermore, we found significant between-treatment differences for biomarkers of oxidative stress (ie, 8-hydroxy-2-deoxyguansine, malondialdehyde, iso-prostaglandin F2α, and superoxide dismutase) (Figure V in the online-only Data Supplement) and inflammation (ie, soluble CD40 ligand, interleukin-1β, and C-reactive protein) (Figure VI in the online-only Data Supplement). Last, levels of insulin, glucose, and homeostatic model assessment of insulin resistance

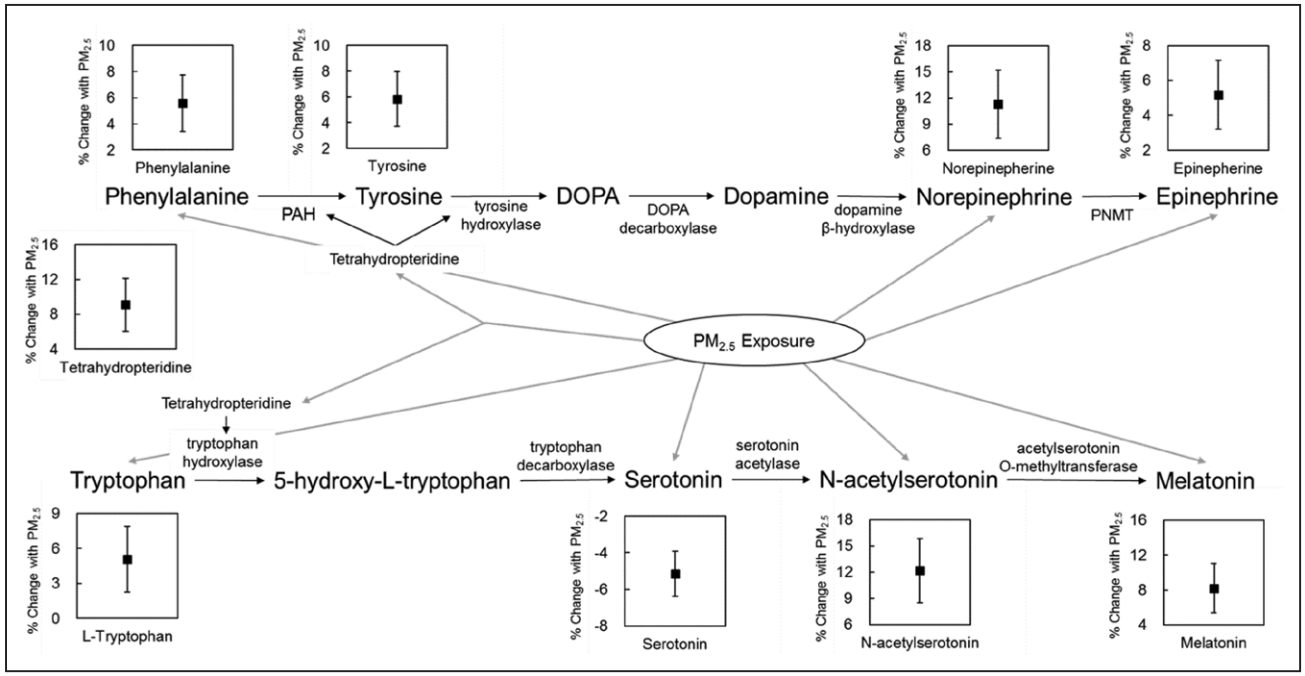

Figure 2. $PM_{2.5}$ exposure and increased synthesis of catecholamine and melatonin.
The y axes represent percentage changes of each metabolite associated with a per 10 μg/m³ increase of $PM_{2.5}$ exposure. DOPA indicates dihydroxyphenylalanine; PAH, phenylalanine hydroxylase; PM, particulate matter; $PM_{2.5}$, particulate matters with aerodynamic diameters ≤2.5μm; and PNMT, phenylethanolamine *N*-methyltransferase.

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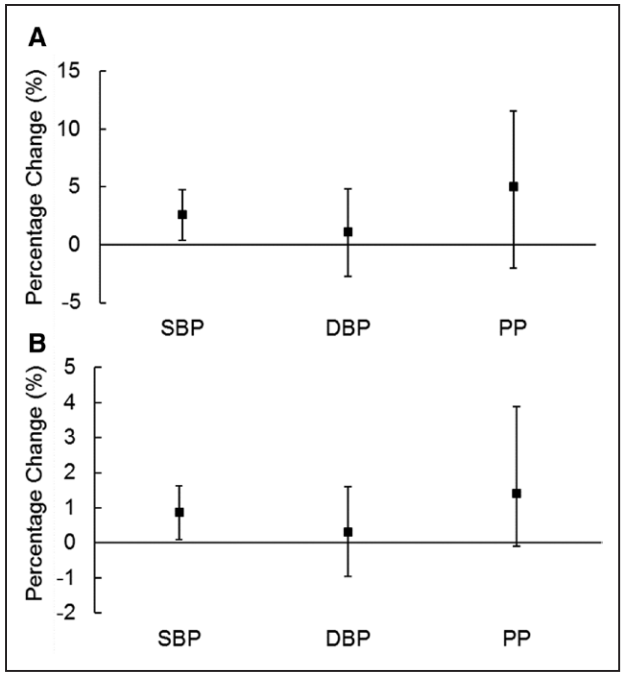

Figure 3. Percentage changes and 95% confidence intervals of blood pressure between sham and real purification (A) or with a per 10 μg/m³ increase of $PM_{2.5}$ exposure (B).

DBP indicates diastolic blood pressure; SBP, systolic blood pressure; $PM_{2.5}$, particulate matters with aerodynamic diameters ≤2.5 μm; and PP, pulse pressure.

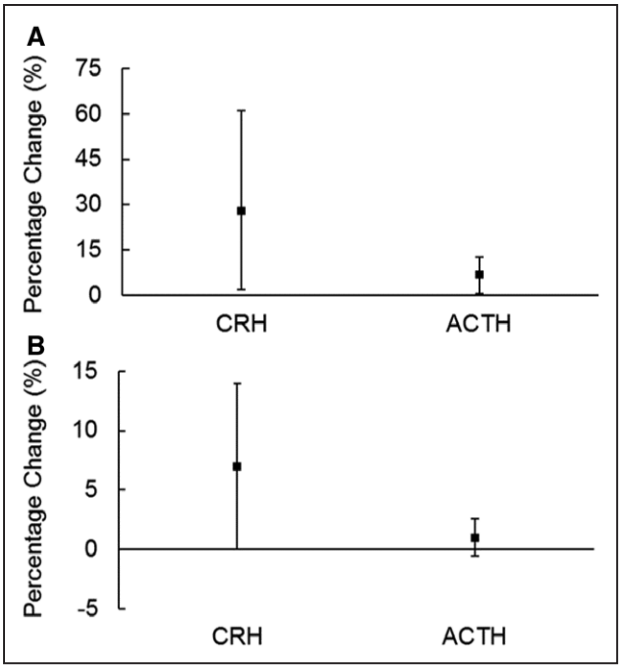

Figure 4. Percentage changes and 95% confidence intervals of corticotropin releasing hormone and adrenocorticotropic hormone between sham and real purification (A) or with a per 10 μg/m³ increase of $PM_{2.5}$ exposure (B).

ACTH indicates adrenocorticotropic hormone; CRH, corticotropin releasing hormone; and $PM_{2.5}$, particulate matters with aerodynamic diameter ≤2.5 μm.

were significantly higher during the sham purification, and, with the exception of insulin, they were also associated with $PM_{2.5}$ as a continuous exposure (Figure VII in the online-only Data Supplement). For these biomarkers, we did not identify any meaningful effects in relation to dormitories and study periods, or significant effect modification by sex.

DISCUSSION

To the best of our knowledge, this is the first study that used the untargeted metabolomics approach to investigate human global metabolic changes in relation to changes in ambient PM exposures. In this randomized, double-blind, crossover trial, we found marked changes in serum metabolites, including hormones, glucose, amino acids, and lipids, in association with higher $PM_{2.5}$ exposure. Consistent with previous publications from us[29] and other groups in China,[7,30] we also observed increases in blood pressure and levels of biomarkers of inflammation and oxidative stress in relation to higher $PM_{2.5}$.

Results from this clinical trial suggest that the human central nervous system reacts to changes in PM exposures. We observed increases in glucocorticoids, ACTH, and CRH in relation to higher PM exposure, suggesting activation of the hypothalamus-pituitary-adrenal (HPA) axis. A study in animals also showed increases of CRH and glucocorticoids after acute exposures to $PM_{2.5}$.[11] It is possible that, in the response to inhalation of PM, the hypothalamus releases CRH, which stimulates the anterior pituitary gland to release ACTH into the circulation. ACTH in turn targets the adrenal cortex and stimulates the synthesis and release of glucocorticoids, mainly cortisol and cortisone. These changes may have several physiological ramifications. Glucocorticoids elevate blood pressure by increasing cardiac output, constricting blood vessels, and inducing sodium and fluid retention.[31] Therefore, the HPA axis activation and increase in glucocorticoids may explain in part the observed higher blood pressures associated with PM exposures. In this study, we observed significant associations of $PM_{2.5}$ with SBP, which might reflect increased cardiac output in response to higher PM exposure; we only observed a positive but statistically insignificant trend for DBP, which mainly reflects vasoconstriction. These observations are consistent with some but not all the previous studies.[15,29,32–34] In addition to its physiological effects on blood pressure, glucocorticoids may also affect energy metabolism by increasing metabolic rate and energy expenditure, increasing blood glucose levels, and enhancing lipolysis, lipid oxidation, proteolysis, and insulin resistance.[35–39] In the present study, we also consistently found that higher $PM_{2.5}$ exposure was

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associated with increased levels of homeostatic model assessment of insulin resistance, glucose, fatty acids, acylcarnitines, and amino acids.

Besides glucocorticoids, our data also suggest that norepinephrine and epinephrine, 2 main catecholamines released by the sympathetic-adrenal-medullary axis, increased in association with PM exposure. The synthesis and secretion of catecholamine in the adrenal medulla is regulated by preganglionic sympathetic neurons. In addition, glucocorticoids from the HPA axis could also modulate catecholamine synthesis by inducing phenylethanolamine *N*-methyltransferase.[40,41] Epinephrine can activate the HPA axis by stimulating ACTH secretion from the pituitary gland.[42] Indeed, our data suggest correlated changes of cortisol, epinephrine, and norepinephrine in relation to changes in $PM_{2.5}$ exposure level (Figure VIII in the online-only Data Supplement). By binding to various cellular receptors, epinephrine and norepinephrine may induce profound metabolic changes via increased glycolysis and lipolysis, hypertensive changes via arterial smooth muscle constriction and increased cardiac output,[43] and proinflammatory changes via increased expression of proinflammatory cytokines.[44]

Last, our results suggest that PM exposure may stimulate the secretion of melatonin, a hormone that regulates circadian cycle and has multiple cardioprotective properties.[45] It has also been suggested that melatonin has antihypertensive effects probably because of its anti-inflammatory and reactive oxygen species scavenging properties, and its capability in improving endothelial function and interacting with central and peripheral nervous system.[46] Melatonin was synthesized from serotonin with *N*-acetylserotonin as an intermediate product. The secretion of melatonin in the pineal body is regulated by norepinephrine via β-adrenergic receptors.[47] Therefore, the changes of melatonin in response to PM exposure level may be influenced by the activation of the HPA and sympathetic-adrenal-medullary axes.

In addition to changes in the central nervous system, we observed higher lysolipids in association with higher PM exposure, which may be the result of membrane hydrolysis. Lysolipids, including lysophosphatidylcholine and lysophosphatidylethanolamine, are important signaling molecules by interacting with G-protein–coupled receptors in various cells and tissues. They can modulate cellular activities such as cellular permeability, apoptosis, and cell proliferation and migration.[48] In addition, lysophosphatidylcholine may induce production of mitochondria reactive oxygen species, upregulate the levels of adhesion molecules, and stimulate secretions of inflammatory chemokines and superoxide anion; these changes may, in turn, lead to endothelial cell activation and vascular endothelial inflammation responses.[49–52] We consistently found increments of inflammatory and oxidative stress biomarkers in association with higher PM exposure. These biological changes may be responsible in part for the adverse cardiovascular effects caused by PM exposure.

One important strength of the current study is that we tested a very feasible strategy to reduce indoor air pollution level among a free-living population. It builds on a previous study from us that demonstrated clear cardiopulmonary benefits following 48 hours of consecutive indoor air purification among college students.[29] Although it remains to be examined whether the observed health benefits could be generalized to other populations or be translated to long-term health benefits, this study offers a very practical way to mitigate some of the adverse health effects for residents in areas with relatively high air pollution.

Our study also has several limitations. First, for outdoor $PM_{2.5}$ concentration, we had to rely on fixed-site monitoring in the campus or in nearby stations, which inevitably led to measurement errors. However, these measurement errors should be nondifferential with respect to both study periods and may cause underestimates in our results.[53] Second, we did not collect blood samples at enrollment and thus were unable to specifically exclude potential impacts from daily variation. However, to a certain extent, this concern could be mitigated by the crossover study design in which all participants started and ended treatments at the same time of the same day within a relatively short study period of 4 weeks. Furthermore, our sensitivity analyses did not find any meaningful period effects.

In this study, we used $PM_{2.5}$ as a marker for overall PM exposure. $PM_{2.5}$ has been used as the PM exposure metric in numerous previous studies.[1,4,26,29] However, because we did not collect data on size-fractioned PM, PM constituents, or gaseous pollutants, we could not attribute our findings solely to $PM_{2.5}$. We do not believe the results could be explained by changes in gaseous pollutants or the interactions between particles and gases because the purifier was designed to remove particles only. However, we cannot exclude the possibility that coarse PM, ultrafine particles, or specific PM components (eg, lipopolysaccharide, metals) may contribute to our observations. In particular, we found higher levels of insulin and ACTH levels during the sham purification group without a statistical association with $PM_{2.5}$ per se, suggesting potential effects attributable to correlated PM metrics.

In summary, this first-ever randomized, double-blind, crossover trial of metabolomics analysis on particulate air pollution demonstrated potential metabolic effects following reduction in short-term PM exposure. Our analyses suggest significant changes in the HPA and sympathetic-adrenal-medullary axes, and changes in glucose, amino acids, and lipids, as well, in relation to changes in PM exposure level. These novel findings provide insights into the potential mechanisms of the adverse health effects that have been found to be associated with $PM_{2.5}$ exposure. Future studies should ex-

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amine whether these health benefits from short-term air purification could be maintained and translated into improved health in the long run.

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DISCLOSURES

None.

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FOOTNOTES

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