



# Inflammatory cytokines and DNA methylation in healthy young adults exposure to fine particulate matter: A randomized, double-blind crossover trial of air filtration



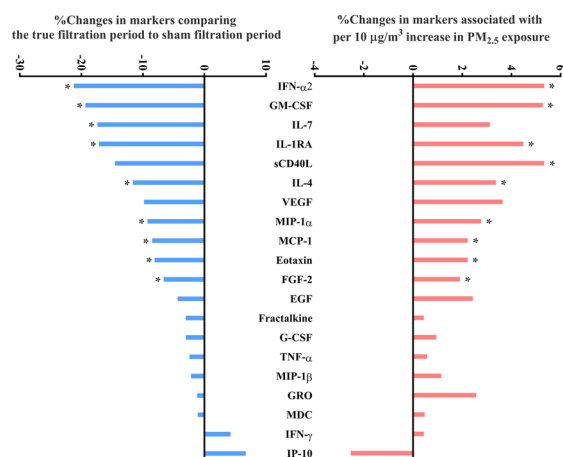
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## GRAPHICAL ABSTRACT



**Abbreviations:** PM<sub>2.5</sub>, Particulate matter with an aerodynamic diameter of 2.5 µm or less; DNAm, DNA methylation; IL, Interleukin; MCP, Monocyte chemoattractant protein; MDC, Macrophage-derived chemokine; MIP, Macrophage inflammatory protein; GRO, Growth-related oncogene; IP, Interferon gamma-induced protein; EGF, Epidermal growth factor; TGF, Transforming growth factor; FGF, Fibroblast growth factor; VEGF, Vascular endothelial growth factor; G-CSF, Granulocyte colony-stimulating factor; GM-CSF, Granulocyte-macrophage colony-stimulating factor; TNF, Tumor necrosis factor; IFN, Interferon; sCD40L, Soluble CD40 ligand; Flt3L, Fms-like tyrosine kinase receptor-3 ligand protein

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## ABSTRACT

Benefits of indoor air filtration in heavily polluted areas are not fully understood. This study aims to examine whether short-term air filtration intervention could attenuate the hazards from acute exposure to fine particulate matter (PM<sub>2.5</sub>), and investigate the potential impact on inflammatory cytokines and DNA methylation. A randomized, double-blind crossover trial of true or sham indoor air filtration was conducted among 29 healthy young adults in Beijing, China. Each episode covered a typical air pollution wave, and 38 cytokines and DNAm of 20 genes were measured at 3 time points: pre-smog, during smog, and post-smog. Linear mixed-effect models were used to evaluate the associations. The indoor PM<sub>2.5</sub> concentration with true filtration was 67.8 % lower than sham filtration (13.8 µg/m<sup>3</sup> vs. 42.8 µg/m<sup>3</sup>). Air filtration was significantly associated with the decreases in 9 cytokines, from 6.61 % to 21.24 %. PM<sub>2.5</sub> exposure was significantly associated with elevated levels of 9 cytokines and changed methylation at 7 CpG sites. Notably, PM<sub>2.5</sub> was significantly associated with GM-CSF, sCD40L, MCP-1, and FGF-2, as well as methylation in corresponding genes, but no mediation effect was observed. This trial suggested that indoor air filtration might attenuate the adverse effects of PM<sub>2.5</sub> exposure through changing cytokines and DNAm.

## 1. Introduction

Short-term and long-term exposure to ambient particulate matter with an aerodynamic diameter of 2.5 µm or less (PM<sub>2.5</sub>) is causally associated with increased cardiovascular disease (CVD) mortality and morbidity (Brook et al., 2010; Liu et al., 2019; Newby et al., 2015). Owing to rapid economic development, the levels of ambient air pollution are extremely high in developing countries. In the Beijing-Tianjin-Hebei region of China, the annual average PM<sub>2.5</sub> concentration was 64 µg/m<sup>3</sup> in 2017 (Ministry of Ecology and Environment, 2018), which is more than six times the value indicated by World Health Organization (WHO) air quality guideline (10 µg/m<sup>3</sup>) (WHO, 2006).

Given that there is a long way to reduce the level of ambient particulate matter in China, and that people typically spend approximately 85 % of their time indoors (Cui et al., 2018), indoor air filtration has been proposed as a practical way to reduce personal exposure to PM<sub>2.5</sub>. Several studies have reported the effect of indoor air filtration on cardiovascular health. These studies have generally reported inconsistent results. A study among 70 adults found that a single overnight residential air filtration was associated with a decrease in thrombogenic marker vWF but no relationship with sCD62P and inflammatory marker IL-6 (Cui et al., 2018). Another earlier study conducted among 35 students in Shanghai found that 48-h dormitory intervention using a portable air cleaner was associated with reduced levels of inflammatory (MCP-1, and IL-1β) and thrombogenic biomarkers (sCD40L) in healthy young adults (Chen et al., 2015).

Although the exact biological mechanisms by which PM<sub>2.5</sub> exerts adverse health effects are not fully understood, systemic inflammation and DNA methylation (DNAm) may result in dysfunctional endothelium and play important roles in regulating atherogenesis and thrombosis (Brook et al., 2010; Esposito et al., 2014; Minelli et al., 2011; Moen et al., 2015). Short-term PM<sub>2.5</sub> exposure has been shown to elevate the levels of cytokines involved in inflammation (Lei et al., 2019; Pope et al., 2016; Rich et al., 2012; Wang et al., 2018; Wu et al., 2016; Yue et al., 2019), but the cytokines are limited. Epidemiological studies have found that exposure to metal particles (Wu et al., 2017) and air pollution (Chen et al., 2016; Gruzieva et al., 2017; Zhang et al., 2019) could lead to alterations in DNAm. Ambient pollutant has been related to hypomethylation in the genes involved in antioxidant defense (Gruzieva et al., 2017), inflammation (Zhang et al., 2019), and long interspersed nucleotide element 1 (*LINE-1*) (Chen et al., 2016). However, little evidence was obtained from studies that focused on the effects of air pollution on DNAm and their encoding proteins simultaneously.

A randomized crossover trial was conducted to explore potential benefits of short-term indoor air filtration intervention during typical air pollution waves in Beijing, China, and to investigate potential roles of inflammatory proteins and methylation in corresponding genes in

PM<sub>2.5</sub> exposure-related adverse health effects.

## 2. Materials and methods

## 2.1. Study design and participants

This randomized crossover trial was conducted between December 2017 and April 2018 in Beijing, China. 32 healthy college students were recruited from 9 dormitory rooms and randomized into 2 groups of dormitories to receive either true or sham air filtration treatment, followed by a washout phase more than 2 weeks; then, the treatment was exchanged (see Fig. S1). Both the treatment episodes covered a process of a typical air pollution wave. Ambient PM<sub>2.5</sub> concentration exceeded 75 µg/m<sup>3</sup>, which also indicated an air quality index greater than 100 and different levels of air pollution, for 2 or more consecutive days, and this was defined as an air pollution wave (Huang et al., 2018). Air quality forecast information was obtained on the following days on the website (<http://aqicn.org/city/beijing/>) to schedule treatment and health examinations. All treatments were performed from the before to after typical air pollution waves (Fig. S1), and visits were conducted 3 times for each episode: pre-smog (baseline), during-smog (first follow-up), and post-smog (second follow-up).

During the treatment episodes, a commercially available air filtration device (X80, 352 Co, Beijing, China) was placed in the center of the dormitory room. The participants lived in 9 dormitories in 2 adjacent buildings without cooking or smoking inside. Each dormitory was about 15 m<sup>2</sup> with 2–4 participants, and the participants' positions were near the 4 corners of dormitories. The true air filtration device consisted of a prefilter, an active carbon filter, and a high-efficiency particulate arrestance (HEPA) filter, which were placed inside the shell of device. In contrast, all 3 filters were removed for the sham air filtration device and the removal of the filters may not influence the appearance of air filtration. In addition, the true airflow was set as 120 m<sup>3</sup>/h and the sound level was less than 40 dB at this airflow rate. The appearance, with noise, and airflow kept the same between the filtered and sham episodes. All participants were encouraged to stay in their dormitories with windows and doors tightly closed throughout the study period, whereas unavoidable outdoor activities, including attending classes and dining in school canteens, were allowed. All research staffs were also blinded to group assignments.

Healthy participants were recruited in Peking University, located in the central urban area of Beijing, where the study was conducted. The inclusion criteria were no smoking history, no smoker roommates, no physician-diagnosed chronic cardiopulmonary diseases, and nonobese. The exclusion criteria were acute infection or medication used for a month and leaving Beijing during the study (including washout phases). Each participant provided written informed consent before the study began. This study was registered at ClinicalTrials.gov with an identifier

NCT03500614 and was approved by the Institutional Review Board of Peking University Health Science Center (No. 00001052-17090)

## 2.2. Exposure measurements

An aerosol spectrometer TSI8530 (TSI, St. Paul, Minnesota, USA) was used for monitoring the real-time indoor PM<sub>2.5</sub> concentration after the baseline health measurements to post-air-pollution-wave health measurements each filtration period. The component “HEPA zero filter” (TSI, St. Paul, Minnesota, USA) was used for zero-point calibrations before each measurement which using a gravimetric method by co-sampling the PM<sub>2.5</sub> mass on 37 mm polytetrafluoroethylene (PTFE) filters (SKC, PA, USA) with a sampling system (2.5- $\mu$ m personal environmental monitor and AirChek XR5000, SKC, PA, USA). Meanwhile, the real-time indoor temperature and relative humidity measurements were conducted using a Temp. Datalogger (model 8828, AZ Instrument Corp, Taiwan). The monitors and sampling system were installed at least 1 m away from the air filtration device and approximately 1.5 m above the ground in each dormitory.

Hourly outdoor PM<sub>2.5</sub> concentration data from 12 fixed-site monitoring stations across Beijing were obtained. Time-location questionnaires were recorded every half-hour by all participants. The time-weighted personal PM<sub>2.5</sub> during each treatment episode was calculated by matching the time-location information and corresponding PM<sub>2.5</sub> concentration data, including both indoor and outdoor monitoring results.

## 2.3. Health measurements

Health measurements were conducted at similar time points on every visit morning to minimize the impact of diurnal variation. To be specific, the “pre-air-pollution-wave” measurement was done before the air pollution waves as the baseline measurement. The “during-air-pollution-wave” measurement was conducted during the air pollution waves following 2 or more satisfactory air polluted days with ongoing treatment. The “post-air-pollution-wave” measurement was done after the air pollution waves, lasting at least 2 days with low air pollution levels (see Fig. S1). Each health measurement was accomplished in the consistent order of blood collection, blood pressure (BP) measurement, fractional exhaled nitric oxide (FeNO), and lung function detection.

## 2.4. Biomarker measurements

Fasting venous peripheral blood samples were drawn at the same time every visit (around 8:00 a.m.). Blood was centrifuged at 3000 rpm for 10 min and separated into plasma within 45 min after collection. Plasma fractions were aliquoted, coded, and stored at -80°C until analysis.

The focus was mainly on the systematic inflammatory cytokines, and the plasma levels of 38 inflammatory cytokines, including interleukin (IL) 1 $\alpha$ , IL-1 $\beta$ , IL1 receptor antagonist (IL-1Ra), IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17A, Eotaxin, monocyte chemotactic protein 1 and 3 (MCP-1 and MCP-3), macrophage-derived chemokine (MDC), macrophage inflammatory protein-1 alpha and 1 beta (MIP-1 $\alpha$  and MIP-1 $\beta$ ), growth-related oncogene (GRO), interferon gamma-induced protein 10 (IP-10), Fractalkine, epidermal growth factor (EGF), transforming growth factor alpha (TGF- $\alpha$ ), fibroblast growth factor 2 (FGF-2), vascular endothelial growth factor (VEGF), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor alpha and beta (TNF- $\alpha$  and TNF- $\beta$ ), interferon alpha 2 and gamma (IFN- $\alpha$ 2 and IFN- $\gamma$ ), soluble CD40 ligand (sCD40L), and fms-like tyrosine kinase receptor-3 ligand protein (Flt3L), were measured using MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel kits (HCYTMAP-60k-PX 38; Millipore Corporation, Billerica, MA, USA) in 96-well plate format. Assays were conducted

using the MAGPIX platform and the software xPONENT (Luminex Corporation, Austin, TX, USA), according to the instructions described by the manufacturer (at <http://www.merckmillipore.com>). Eighteen markers (IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-3, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17A, Flt3L, MCP-3, TGF- $\alpha$ , and TNF- $\beta$ ) with more than 50 % of the samples below the limit of detection were excluded from the analysis. Markers with concentrations below the LOD were replaced by the 1/2 of the detection limits. All the samples were run in duplicate in each batch.

## 2.5. DNA methylation measurements

Twenty-six participants whose DNA samples met the detection requirements were selected under sham filtration episode to detect the level of DNAm because of a limited budget. DNA isolated from peripheral CD4<sup>+</sup> T-helper cells was used to detect methylation at 254 CpGs located near or within the promoter regions of genes that encoding the analyzed cytokines using Illumina Infinium Human MethylationEPIC Beadchip (850k). All analyses were conducted according to the manufacturer's instructions. Quality control was performed, and CpG sites with detection *P*-value of < 0.05 were kept. Signals were then normalized using the quantile method (Touleimat and Tost, 2012). The level of DNAm at a particular CpG site (also called the methylation beta-value ( $\beta$ )) was calculated by taking the ratio of the methylated intensity (M) to unmethylated intensity (U) signal, using the formula:  $\beta = M / (M + U)$ . A  $\beta$ -value of 0 represents a completely unmethylated CpG site, and a  $\beta$ -value approaching 1 represents a fully methylated CpG site. Probes on sex chromosomes were also removed, as well as those identified as cross-hybridizing with other genomic locations or SNP associated (Pidsley et al., 2016). To assess differences in methylation between groups, the original  $\beta$  values were converted to M-values via the logit transformation. Differentially methylated probes were detected using the R bioconductor limma (3.36.3) package. Probes with an adjusted *P*-value of < 0.05 were considered differentially methylated. The Benjamini–Hochberg method was used to adjust the *P*-value and ensure that the false discovery rate was < 0.05. The corresponding gene list was derived from the gene annotations associated with the probes.

## 2.6. Statistical analysis

The paired Wilcoxon rank sum test was used to compare change of cytokines between true and sham filtration treatments. A subject-mean adjusted linear mixed-effects regression model was applied to evaluate the effects air filtration intervention (dichotomized as “true” or “sham”) on cytokines expression. This model was also applied to evaluate continuous time-weighted personal PM<sub>2.5</sub> concentrations to inflammatory cytokines and DNAm. It accounts for subject-specific differences by subtracting subject-level means, and regressing the deviations from these means on treatment or PM<sub>2.5</sub> (Pope et al., 2016). The subject was included as a random effect, which enabled participants to serve as their own controls in this model and accounted for intraindividual correlations between repeated measurements (Wu et al., 2013). All models were adjusted for sex, age, body mass index (BMI), day of week, length of treatment, indoor temperature, and relative humidity. (Expanded statistical methods in the Supplemental Material.) In addition, we conducted a mediation analysis to estimate and quantify the contribution of DNAm in the candidate gene loci, as a mediator of the effect of PM<sub>2.5</sub> on the cytokine. The approach decomposes the total effect into a direct effect and an indirect effect (i.e. the mediation effect) that acts through a mediator.

We further performed subgroup analysis to estimated gender-specific associations between PM<sub>2.5</sub> and cytokines. In the sensitivity analysis, we treated all cytokine samples below the LOD as missing values in the linear mixed-effects model to explore the stability of associations between cytokine and air filtration intervention and personal PM<sub>2.5</sub>

exposure.

The Benjamini–Hochberg (BH) false discovery rate (FDR) method was used to account for multiple testing, and results with  $P < 0.05$  and  $FDR < 0.10$  were considered statistically significant. We used the existing R “mediation” package created by Imai and colleagues for mediation analysis and other statistical analyses were performed using SAS software (version 9.4; SAS Institute, Cary, NY, USA). The effects of air filtration were presented as the percent changes and 95 % confidence intervals (CIs) in markers comparing the true air filtration treatment with the sham treatment. The effects of  $PM_{2.5}$  were presented as the percent changes (and 95 % CIs) for each cytokine and DNAM for each  $10 \mu g/m^3$  increase in  $PM_{2.5}$  relative to the mean values.

### 3. Results

This study recruited 32 participants and 3 dropped out because of personal reasons, leaving 29 participants in the final analysis. There were 16 males and 13 females with an average age of  $22 \pm 2$  years and a mean body mass index of  $21 \text{ kg/m}^2$ . Participants remained healthy during the whole study.

The time-varying  $PM_{2.5}$  concentration and the time points for blood draws in each visit are illustrated in Fig. 1. The mean outdoor  $PM_{2.5}$  concentration was  $68.0 \mu g/m^3$ , with hourly concentrations ranging from  $3.0$ – $249.0 \mu g/m^3$ . The mean indoor  $PM_{2.5}$  concentration in rooms with sham filtration was  $42.8 \mu g/m^3$ . However, the average indoor  $PM_{2.5}$  concentration in rooms with true filtration was 79.7 % lower than that outdoors, reducing the  $PM_{2.5}$  concentration to  $13.8 \mu g/m^3$ .

Most of the inflammatory cytokines were appreciably decreased in the true filtration episode compared with the sham filtration period from pre-smog to during smog, although a significant reduction was only observed for GRO ( $t = -96.50$ ,  $P = 0.0344$ ). MIP-1 $\alpha$  ( $T = 47.5$ ,  $P = 0.0232$ ) and MIP-1 $\beta$  ( $t = 2.29$ ,  $P = 0.0299$ ) showed a statistically significant increase from during smog to post-smog in the true filtration compared with the sham filtration period (Table S1).

In the subject-mean adjusted linear mixed-effect model analysis, compared with the sham filtration treatment, the true filtration was significantly associated with decreases in 9 circulating markers ( $FDR < 0.10$ ), including IFN- $\alpha 2$ , GM-CSF, IL-7, IL-1RA, IL-4, MIP-1 $\alpha$ , MCP-1, Eotaxin, and FGF-2, with decreases of 21.24 % ( $P = 0.0078$ ),

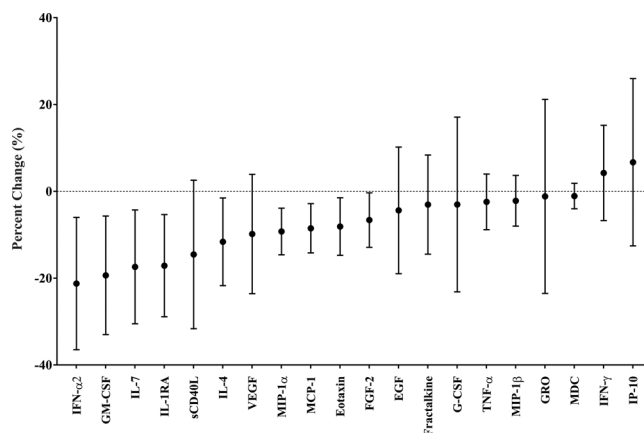


**Fig. 2.** Percent changes in inflammatory cytokines comparing the true filtration period to sham filtration period. The results are presented as percent change and 95 % CIs in each cytokine comparing the true filtration period with the sham filtration period. Estimated effects are derived from the subject-mean adjusted regression, which is adjusted for sex, BMI, day of week, indoor temperature, indoor relative humidity, and length of treatment. Based on estimated values, the most statistically significant inverse associations are on the left and the most positive associations are on the right.

19.35 % ( $P = 0.0068$ ), 17.40 % ( $P = 0.0111$ ), 17.12 % ( $P = 0.0056$ ), 11.62 % ( $P = 0.0270$ ), 9.24 % ( $P = 0.0012$ ), 8.51 % ( $P = 0.0043$ ), 8.11 % ( $P = 0.0189$ ), and 6.61 % ( $P = 0.0421$ ), respectively. The associations were robust after correcting for multiple comparisons (Table S2). Estimated effects were ordered from left to right based on percent change values (Fig. 2).

Fig. 3 presents the associations between time-weighted personal  $PM_{2.5}$  concentrations and 20 analyzed cytokines. A  $10 \mu g/m^3$  increase in time-weighted personal  $PM_{2.5}$  was significantly associated with increases of 5.33 % ( $P = 0.0218$ ), 5.28 % ( $P = 0.0112$ ), 4.48 % ( $P = 0.0127$ ), 5.34 % ( $P = 0.0369$ ), 3.38 % ( $P = 0.0270$ ), 2.77 % ( $P = 0.0008$ ), 2.22 % ( $P = 0.0103$ ), 2.22 % ( $P = 0.0267$ ), and 1.91 % ( $P = 0.0438$ ) in IFN- $\alpha 2$ , GM-CSF, IL-1RA, sCD40L, IL-4, MIP-1 $\alpha$ , MCP-1, Eotaxin and FGF-2, respectively. Notably, we found that the

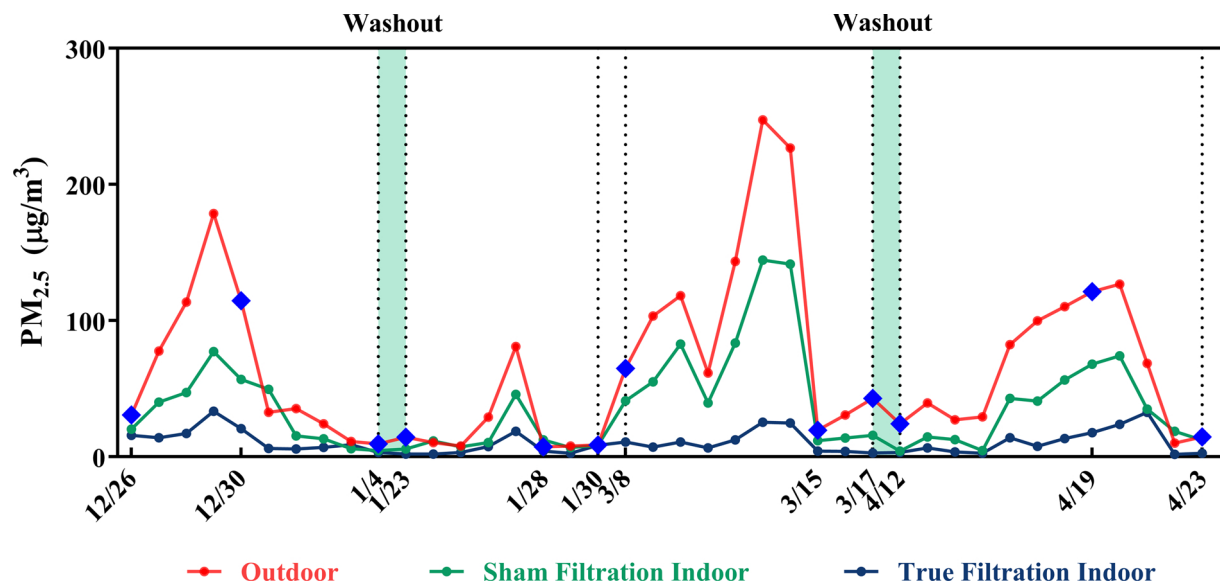


**Fig. 1.** Time-varying  $PM_{2.5}$  concentration in outdoor, sham filtration indoor, and true filtration indoor conditions and the time points of blood draws for each visit.  $PM_{2.5}$  concentrations and blood-draw dates are plotted over study periods. Red line plots indicate outdoor ambient  $PM_{2.5}$  24-h average concentrations based on continuous monitors from nearby environmental monitoring site. Green line plots indicate indoor  $PM_{2.5}$  24-h average concentrations with sham filtration. Blue-black line plots indicate indoor  $PM_{2.5}$  24-h average concentrations with true filtration. Diamonds indicate  $PM_{2.5}$  concentrations at the times of blood draws. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

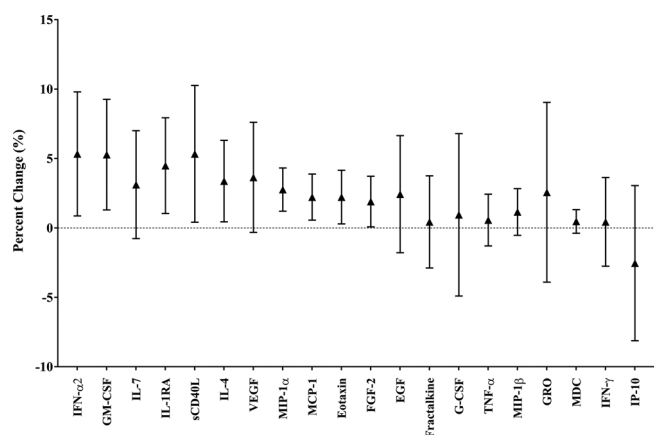


**Fig. 3.** Percent changes in inflammatory cytokines associated with per 10  $\mu\text{g}/\text{m}^3$  increase in time-weighted personal  $\text{PM}_{2.5}$  exposures. The results are presented as percent change and 95 % CIs in each cytokine associated with per 10  $\mu\text{g}/\text{m}^3$  increase in time-weighted personal  $\text{PM}_{2.5}$  exposures relative to the mean. Estimated effects are derived from the subject-mean adjusted regression, which is adjusted for sex, BMI, day of week, indoor temperature, indoor relative humidity, and length of treatment. The order is consistent with Fig. 2.

associations were generally robust after correcting for multiple comparisons (Table S3). The patterns of these associations were highly consistent with those for the air filtration treatment for IFN- $\alpha 2$ , GM-CSF, IL-1RA, IL-4, MIP-1 $\alpha$ , MCP-1, Eotaxin, and FGF-2, with positive associations with  $\text{PM}_{2.5}$  exposure increase and inverse associations with air filtration. The order of markers in Fig. 3 was based on Fig. 2. The associations between  $\text{PM}_{2.5}$  and subjected-mean adjusted values for MCP-1, MIP-1 $\alpha$ , GM-CSF, and FGF-2 were shown in Fig. 4.

As shown in Table 1, Significantly changed methylation was detected at 7 out of 254 CpGs loci in response to the increase in  $\text{PM}_{2.5}$ , and the magnitude of associations varied considerably (-3.97 % to 13.28 %). DNAm levels at 2 CpG sites demonstrated significant inverse associations with  $\text{PM}_{2.5}$ , including cg06218285 (-1.84 %; 95 % CI, -3.45 % to -0.22 %) in the gene body of *CD40* (encoding sCD40L) and cg05440824 (-2.98 %; 95 % CI, -5.63 % to -0.33 %) in the promoter (TSS1500) of *CCL2* (encoding MCP-1 protein). Furthermore, we observed that elevated  $\text{PM}_{2.5}$  was significantly and positively associated with DNAm levels at 5 CpGs loci: cg08686879, cg17547898, cg26596307, cg06830319, and cg01654824, which were annotated to genes *CSF2*, *FGF2*, *CX3CL1*, *CX3CL1*, and *CXCL10*, respectively. DNAm at 4 genes (*CD40*, *CCL2*, *CSF2*, and *FGF2*) and the levels of corresponding encoded cytokines were both significantly associated with  $\text{PM}_{2.5}$  exposure. The associations against per 10  $\mu\text{g}/\text{m}^3$  increase in time-weighted personal  $\text{PM}_{2.5}$  exposures were plotted in Fig. 5. Notably, after correcting for multiple comparisons, the associations between  $\text{PM}_{2.5}$  and methylation at the 7 CpG sites turned to be nonsignificant. Mediation analysis was applied to evaluate the role of DNA methylation as mediator of the association between  $\text{PM}_{2.5}$  and these 4 inflammatory markers. However we did not find any significant mediation effect (data not shown).

The impact of  $\text{PM}_{2.5}$  on inflammatory markers was examined in male and female participants separately in gender-stratified analyses, and the results are summarized in Table S4. Short-term exposure to  $\text{PM}_{2.5}$  was associated with GM-CSF and MIP-1 $\alpha$  in males, and with IFN- $\alpha 2$ , IL-4, MCP-1 and Eotaxin in females. These associations were significant at  $P < 0.05$  but none was significant at  $\text{FDR} < 0.10$ . Overall, in the sensitivity analysis, similar results were obtained for the models that treated all cytokines observations below the LOD as missing observations (Tables S5 and S6).

#### 4. Discussion

In this crossover trial among healthy young adults in Beijing, a

heavily polluted megacity, the potential cardiovascular benefits of using an indoor air filtration device against the hazard of  $\text{PM}_{2.5}$  exposure was investigated, and the potential signal transduction pathway and epigenetic mechanisms were explored. It was observed that short-term exposure to  $\text{PM}_{2.5}$  contributed to changes in inflammatory cytokines and DNAm, even in a young, healthy population who have no pre-existing diseases and are unconfounded by disease progression, and the changes in cytokines could be, to a certain extent, reduced by using an indoor air filtration device. The findings provide mechanistic evidence supporting the hypothesis that elevated  $\text{PM}_{2.5}$  could induce an acute increase of inflammatory cytokines and changes in DNAm that contribute to plaque rupture and thrombosis and, thus, trigger acute CVD events (as shown in Fig. 6).

Systemic inflammation is thought to be a relevant mechanism that links pulmonary  $\text{PM}_{2.5}$  inhalation to adverse cardiovascular effects. The assessment of personal intervention approaches in minimizing the hazards of  $\text{PM}_{2.5}$  is crucially important to public health. In the current study, we found that indoor air filtration led to reduction in almost all of the 20 analyzed plasma inflammatory markers, particularly for 9 inflammatory proteins including IFN- $\alpha 2$ , GM-CSF, IL-7, IL-1RA, IL-4, MIP-1 $\alpha$ , MCP-1, Eotaxin, and FGF-2, but not sCD40L. This non-significant effect on sCD40L conflicts with a similar dormitory-based indoor air purification study in Shanghai, which also reported reduced expression of MCP-1 (Chen et al., 2015). In contrast to the findings from several earlier indoor air filtration studies in regions with cleaner air (Allen et al., 2011; Brugge et al., 2017; Karotki et al., 2013), our study provides evidence to show that health benefits may be more easily achieved in countries with severe air pollution. However, we did not find significant benefits for the remaining markers, some of which were reported to be associated with  $\text{PM}_{2.5}$  exposure in several previous studies (Pope et al., 2016; Wang et al., 2018; Yang et al., 2017). The nonsignificant effects on these markers may be due to the relative small sample size, which make the evaluated effects imprecise.

Short-term exposure to  $\text{PM}_{2.5}$  has been linked with risk factors that may augment susceptibility to cardiovascular mortality or morbidity (Pope and Dockery, 2020), and with a range of circulatory inflammatory markers by which airborne particles initiate adverse biological effects. Many markers associated with  $\text{PM}_{2.5}$  exposure have been used as indicators of adverse health effects after exposure to  $\text{PM}_{2.5}$ , but the results are inconsistent (Yang et al., 2017). Chemokine and colony-stimulating factor could regulate acute inflammatory response by attracting macrophages and monocytes in injured tissues (Kolattukudy and Niu, 2012) and at all stages of atherosclerosis (Phipps, 2000) and stimulating proliferation and migration of effector granulocytes and macrophages (Dougan et al., 2019; Morgan et al., 1976; Wicks and Roberts, 2016). Significant increases in MCP-1, MIP-1 $\alpha$ , and GM-CSF have been associated with  $\text{PM}_{2.5}$  exposure among young adults (Chen et al., 2015; van Eeden et al., 2001; Xu et al., 2019). Similarly, we found that short-term exposure to  $\text{PM}_{2.5}$  was significantly associated with increases in GM-CSF, MIP-1 $\alpha$ , MCP-1, and Eotaxin. Similarly, a previous study by Pope et al., 2016 also observed positive associations between short-term exposure to  $\text{PM}_{2.5}$  and inflammatory markers MIP-1 $\alpha$  and MCP-1. Nevertheless, insignificant increase in GM-CSF and Eotaxin were also reported in that study. The inconsistency of the observations may be partially due to the different background concentration of  $\text{PM}_{2.5}$ .

As a marker of acute platelet activation (Antoniadou et al., 2009), sCD40L is implicated in the pathogenesis of inflammation, thrombosis, and atherosclerosis (Phipps, 2000; Zhang et al., 2015). Positive associations between  $\text{PM}_{2.5}$  and sCD40L have been documented in previous studies (Chen et al., 2015; Pope et al., 2016; Rich et al., 2012). Consistent with the prior studies,  $\text{PM}_{2.5}$  exposure was associated with increase in the level of sCD40L in the present study.

Angiogenesis often accompanies the inflammatory response (Zittermann and Issekutz, 2006). FGF-2 is a potent angiogenic growth factor that involved in vascular remodeling (Bikfalvi et al., 1995) and

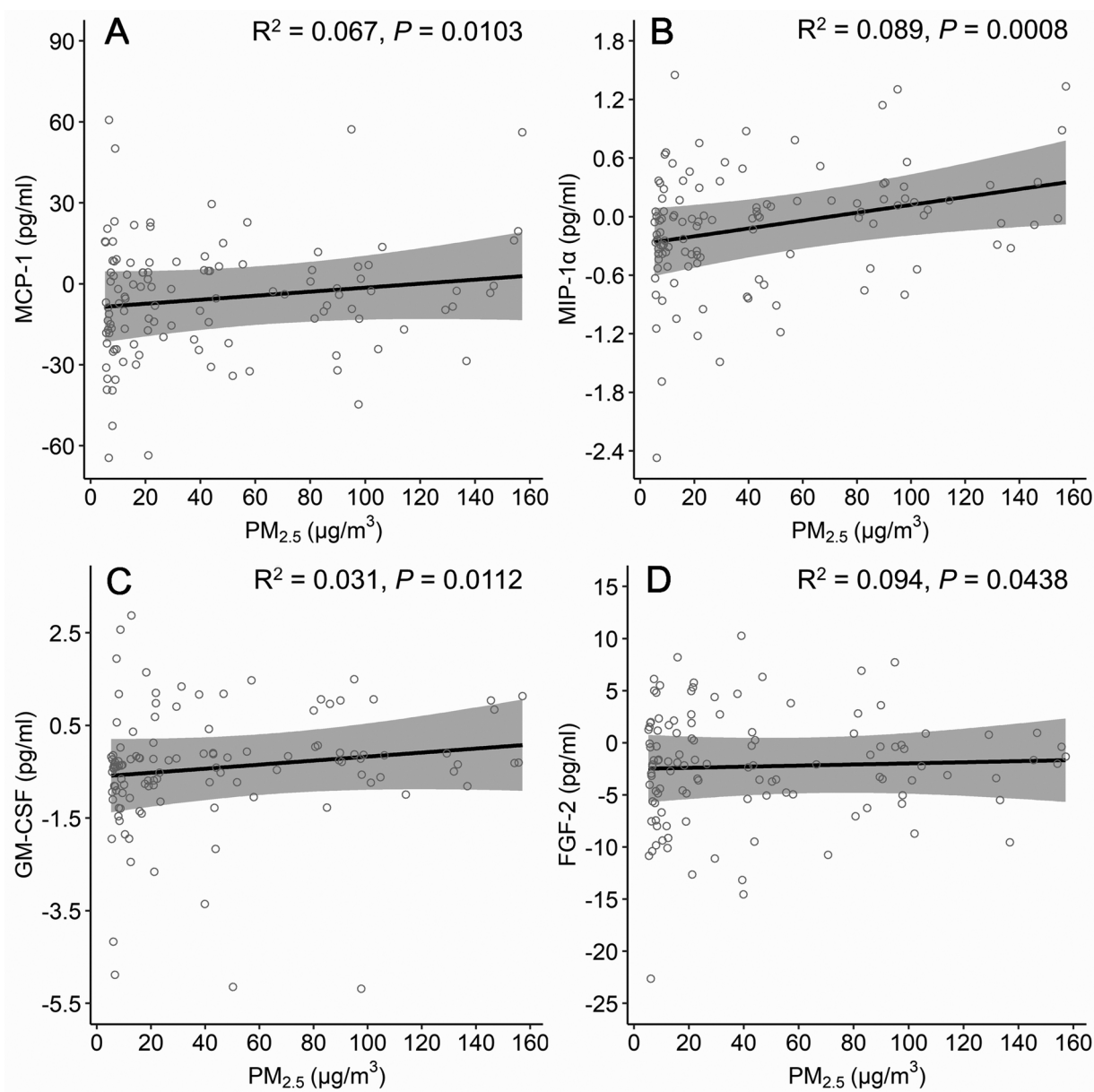


**Fig. 4.** Exposure-effect relationships between time-weighted personal  $PM_{2.5}$  exposures and typical key cytokines. Subject-mean adjusted values for MCP-1 (A), MIP-1 $\alpha$  (B), GM-CSF (C), and FGF-2 (D) are plotted over  $PM_{2.5}$  concentrations, with fitted regression lines, 95 % confidence limits, and 95 % prediction limits using linear mixed-effect model controlling for sex, BMI, day of week, indoor temperature, indoor relative humidity, and length of treatment; this model used time-weighted personal  $PM_{2.5}$  concentrations 24 h prior to blood draws and included subject-mean adjusted values for MCP-1 and MIP-1 $\alpha$  of 29 participants during smog and post-smog of 2 periods.

**Table 1**

Percent changes in DNA methylations associated with per 10  $\mu g/m^3$  increase in time-weighted personal  $PM_{2.5}$  exposures.

| CpG        | Chr   | Gene          | Cytokine    | Percent Change | 95 % CI |        | Relation to Gene | P      | FDR    |
|------------|-------|---------------|-------------|----------------|---------|--------|------------------|--------|--------|
|            |       |               |             |                | Lower   | Upper  |                  |        |        |
| cg08686879 | Chr5  | <i>CSF2</i>   | GM-CSF      | 3.116          | 0.987   | 5.245  | 1stExon          | 0.0095 | 0.9685 |
| cg06218285 | Chr20 | <i>CD40</i>   | sCD40L      | -1.837         | -3.453  | -0.221 | Body             | 0.0378 | 0.9685 |
| cg05440824 | Chr17 | <i>CCL2</i>   | MCP-1       | -2.976         | -5.625  | -0.326 | TSS1500          | 0.0395 | 0.9685 |
| cg17547898 | Chr4  | <i>FGF2</i>   | FGF-2       | 3.326          | 0.299   | 6.353  | Body             | 0.0436 | 0.9685 |
| cg26596307 | Chr16 | <i>CX3CL1</i> | Fractalkine | 2.065          | 0.363   | 3.768  | Body             | 0.0275 | 0.9685 |
| cg06830319 | Chr16 | <i>CX3CL1</i> | Fractalkine | 7.890          | 0.500   | 15.279 | TSS1500          | 0.0494 | 0.9685 |
| cg01654824 | Chr4  | <i>CXCL10</i> | IP-10       | 3.964          | 1.566   | 6.362  | 5'UTR;Body       | 0.0041 | 0.9685 |

Note: Chr, Chromosome; Body, gene body; TSS200, within 200 bps from transcription start site; TSS1500, within 1500 bps from transcription start site; UTR, untranslated region. FDR, false discovery rate.

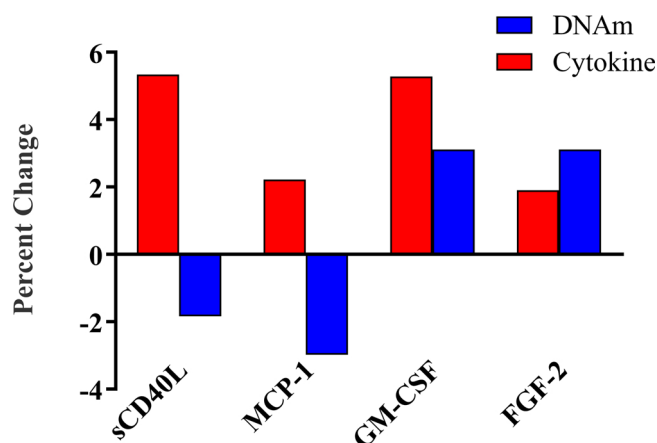


Fig. 5. Percent Changes in cytokines and corresponding DNAm associated with per 10  $\mu\text{g}/\text{m}^3$  increase in time-weighted personal  $\text{PM}_{2.5}$  exposures.

the aggravation of inflammation (Zittermann and Issekutz, 2006). Short-term exposure to  $\text{PM}_{2.5}$  induced vascular remodeling in mice (Yue et al., 2019) but inhibition of angiogenesis in human umbilical vein endothelial cells (Zhu et al., 2019). We found that short-term exposure to  $\text{PM}_{2.5}$  induced significant increase in plasma FGF-2. Similar to our finding, a panel study reported that  $\text{PM}_{2.5}$  could elevate the level of FGF-2, but not statistically significant (Pope et al., 2016).

Many studies have examined the association between short-term exposure to air pollution and inflammatory markers (Brook et al., 2010), but few studies have focused on IFN- $\alpha$ 2, IL-4, and IL-1RA. IFN- $\alpha$ 2, a member in the type I IFNs family, is an effective immune stimulator that enhance T cell- and natural killer (NK) cell-mediated cytotoxicity and has been used in the treatment of some viral infections (Ng et al., 2016). IL-4 is known to increase IL-6 levels and influence barrier function of vascular endothelial cell (Skaria et al., 2016). It has been suggested that PM exposure contributes to the increase in IL-4 (Corradi et al., 2010). However, IL-1RA is a potent anti-inflammatory

mediator which known to rise with the increase of IL-1 and a marker of the initial cytokine response by macrophages to particulate matter exposure (Rajagopalan and Brook, 2012). In the current study, short-term  $\text{PM}_{2.5}$  exposure was associated with the elevated levels in IFN- $\alpha$ 2, IL-1RA, and IL-4. Several reports have shown similar results that short-term exposure to particulate matter increased the levels of IL-1RA and IL-4 (Calderon-Garciduenas et al., 2013; Kumarathasan et al., 2018; Lucht et al., 2019). However, nonsignificant or inverse associations between  $\text{PM}_{2.5}$  exposure and these 3 markers were also reported (Gao et al., 2020; Pope et al., 2016; Shakyia et al., 2019).

The biological pathways that  $\text{PM}_{2.5}$  led to the expression of these markers are still not fully elucidated (Munzel et al., 2017). DNAm has been proposed as one of the potential mechanisms through which  $\text{PM}_{2.5}$  induced adverse health outcomes (Zhong et al., 2017). Our study identified 7 CpG sites associated with  $\text{PM}_{2.5}$  exposure based on  $P < 0.05$ , though with  $\text{FDR} > 0.10$ . 5 CpG sites showed increased methylation and 2 (cg06218285, and cg05440824) showed decreased methylation after exposure to  $\text{PM}_{2.5}$ . Specifically, cg06218285 was annotated to the gene body of *CD40*, which encodes sCD40L. In accordance with our finding, a previous study in Shanghai reported that  $\text{PM}_{2.5}$  was significantly associated with hypomethylation in *CD40LG* (Chen et al., 2016). In contrast to the study by Chen et al. (Chen et al., 2016), we found an inverse association between  $\text{PM}_{2.5}$  and methylation at cg05440824 which locates in the promoter of *CCL2*. Methylation in promoter region has been shown to result in transcriptional repression, but the influence of gene-body DNAm remains mixed (Yang et al., 2014). Furthermore, we reported significant positive associations between  $\text{PM}_{2.5}$  exposure and methylation in *CSF2*, *FGF2*, *CX3CL1*, and *CXCL10*, which have not been investigated against air pollution exposure in previous studies. Although it was not observed that methylation in any locus could mediate the effects of  $\text{PM}_{2.5}$  on related proteins, our findings warrant further researches on mechanisms of methylation that may play a role in the induction of acute inflammation.

Although sex has not been definitively identified as an effect modifier, we still found that the associations between  $\text{PM}_{2.5}$  and

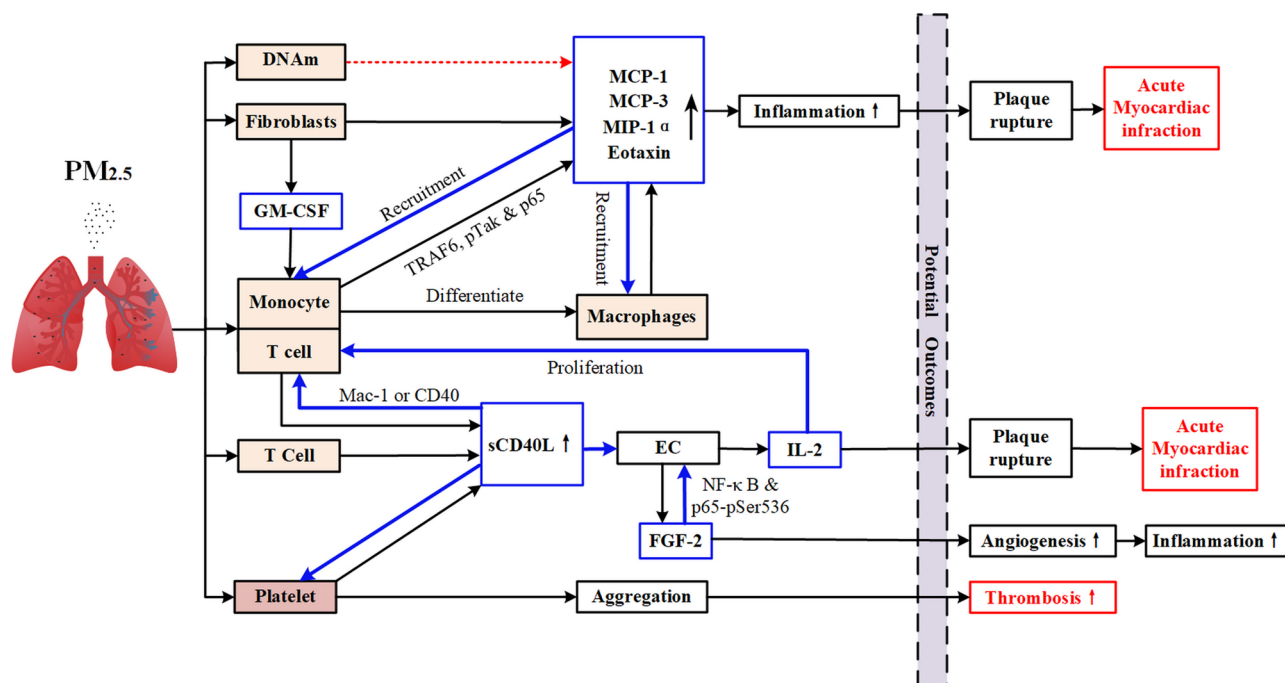


Fig. 6. Schematic diagram of the hypothesized mechanism that links  $\text{PM}_{2.5}$  exposure to observed effects and cardiovascular events. Inhaled  $\text{PM}_{2.5}$  triggers the release of cytokines in the blue box, which are involved in inflammation, coagulation, and angiogenesis. These responses may contribute to the acute cardiovascular events.  $\text{PM}_{2.5}$ , particulate matter with an aerodynamic diameter of 2.5  $\mu\text{m}$  or less; EC, the endothelial cell. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

cytokines varied between male and female in the stratified analyses. There were significant positive association between PM<sub>2.5</sub> and IFN- $\alpha$ 2, IL-4, MCP-1 and Eotaxin only in females, and significant positive associations between PM<sub>2.5</sub> and GM-CSF and MIP-1 $\alpha$  only in males. However, two previous studies have reported that the inflammation effects of short-term and long-term PM exposures are more pronounced in men (Allen et al., 2011; Hoffmann et al., 2009). The inconsistent findings may be due to the differences in PM<sub>2.5</sub> characteristic and study participants.

In view of these changes resulting from acute exposure to PM<sub>2.5</sub> in young adults, which, if sustained, could lead to the development of chronic morbidity (e.g., atherosclerotic cardiovascular) and the precipitation of acute CVD events (e.g., stroke and acute MI) in their later lives, it would be of great public health interest if air filtration intervention could attenuate changes in inflammatory cytokines that link PM<sub>2.5</sub> exposure with disease.

This study has strengths in investigating the effect of air filtration intervention. First, in this randomized crossover design based on true and sham air filtration, self-contrast in exposure and no need for introducing an external reference population were obtained. Second, this study recruited young, healthy adults on campus, and the results could reflect the direct effects of PM<sub>2.5</sub> and avoid bias resulting from disease exacerbation. The dormitories also provided an easily controlled environment. Third, the study was conducted during typical air pollution waves in Beijing, which made it possible to explore the effect of air filtration during extreme air pollution conditions and provide a natural wide range of exposure levels.

The study also has several limitations. First, the relative small sample size, which may limit the power and precision. Second, the outdoor PM<sub>2.5</sub> concentration in this study was based on fixed-site monitoring in nearby stations, and, although this is widely used (Hutcheon et al., 2010), personal PM<sub>2.5</sub> exposure was calculated based on time–location information but not measured by personal particulate monitors, which inevitably resulted in measurement errors. These errors could lead to underestimates of the results. Third, gaseous pollutants were not included in this study. In addition, with the reduction of PM<sub>2.5</sub> during air filtration episode, the chemical compositions such as the semi-volatile organic species (SVOCs) in the remaining PM<sub>2.5</sub> may also change and contribute to the alteration of the markers. Further studies are encouraged to investigate this issue in specific detail. Fourth, the effect of air filtration on the DNAm was not analyzed because of budget limitations.

## 5. Conclusions

This study demonstrated that acute PM<sub>2.5</sub> exposure can induce unfavorable effects in inflammatory cytokines and DNAm in healthy, young adults and that the changes in cytokines can be attenuated by air filtration. It suggests that air filtration is a relatively affordable and practical way to protect public health against PM<sub>2.5</sub>-exposure-related hazards, even in extreme air pollution areas. Although these results need validation with additional research, our findings may contribute to the understanding of the associations between PM<sub>2.5</sub> and acute cardiovascular events. Further studies with larger sample sizes are required to shed light on the precise mechanisms of PM<sub>2.5</sub>-induced hazards and the long-term efficacy of air filtration.

## CRedit authorship contribution statement

**Yanyan Sun:** Software, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Jing Huang:** Conceptualization, Methodology, Supervision, Writing - review & editing, Funding acquisition. **Yan Zhao:** Investigation, Data curation, Resources, Project administration. **Lijun Xue:** Investigation, Resources, Validation. **Hongyu Li:** Investigation, Data curation. **Qisijing Liu:** Investigation, Data curation. **Han Cao:** Software, Formal analysis.

**Wenjuan Peng:** Software, Visualization. **Chunyue Guo:** Data curation, Formal analysis. **Yunyi Xie:** Data curation, Visualization. **Xiaohui Liu:** Data curation, Visualization. **Bingxiao Li:** Data curation, Software. **Kuo Liu:** Formal analysis, Visualization. **Shaowei Wu:** Methodology, Conceptualization, Writing - review & editing, Funding acquisition. **Ling Zhang:** Methodology, Supervision, Writing - review & editing, Funding acquisition.

## Declaration of Competing Interest

The authors declare no actual or potential competing conflicts of interests.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jhazmat.2020.122817>.

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