

The Role of Melatonin in Pathophysiologic Responses to Air Pollution Exposure

by

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Dissertation submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Environment
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ABSTRACT

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Abstract

It is widely accepted that the pathophysiologic pathways linking air pollution exposure and adverse health effects are via the augmentation of oxidative stress and inflammation in the respiratory tract and the circulatory system. Melatonin is a potent antioxidant and anti-inflammatory molecule and may thereby affect individuals' biological responses to air pollution exposure. This dissertation aims to investigate the role of melatonin in pathophysiological responses to air pollution exposure.

In Aim 1 of this dissertation research, a method was developed to simultaneously measure urinary concentrations of 6-sulfatoxymelatonin (aMT6s) and 8-hydroxy-2'-deoxyguanosine (8-OHdG). As a major metabolite of melatonin excreted in the urine, aMT6s has been widely used as a surrogate of circulating melatonin. Urinary 8-OHdG, a stable product of DNA oxidative damage, has been used as an oxidative stress biomarker. This new method is expected to have important applications in biomedical and environmental health studies involving the oxidative stress pathophysiological pathway.

In Aim 2 of this dissertation research, the role of melatonin in oxidative stress responses to air pollution exposure was examined. Stored urine samples collected from 159 healthy adults, and their personal air pollution exposure data were used. These urine samples were analyzed for aMT6s and 8-OHdG; and statistical analyses were conducted to examine the relationships among aMT6s, 8-OHdG and another previously

measured urinary oxidative stress biomarker, malondialdehyde (MDA), and pollutant exposures. The results of this analysis suggest the need for controlling for aMT6s as a confounder in using urinary 8-OHdG and MDA as biomarkers of oxidative stress related to short-term air pollution exposure.

In Aim 3 of this dissertation research, the role of melatonin in inflammatory responses to air pollution exposure was examined. Blood inflammatory cytokines and urinary aMT6s were measured in 53 healthy adults three times within 2 consecutive months. Personal air pollution exposure was calculated prior to biospecimen collections. The study found that concentrations of proinflammatory cytokines were significantly and negatively associated with O₃ exposures averaged over the preceding 12 hours while significantly but positively associated with O₃ exposures averaged over the preceding 2 weeks. These findings suggest that exposure to O₃ for different time durations may affect systemic inflammatory responses in different ways. In addition, the study found that pro-inflammatory responses to O₃ exposure in the preceding 2 weeks may partly result from the depletion of endogenous melatonin by O₃.

In Aim 4 of this dissertation research, the role of melatonin in pathophysiologic and oxidative stress responses to air pollution exposure in asthmatic children was examined. Urine, nasal fluid, and pulmonary physiology data were obtained from 43 asthmatic children four times with a 2-week interval between the consecutive clinic visits. At each visit, pulmonary physiology indicators, comprised of airway mechanics,

lung function, airway inflammation, and asthma symptom scores were measured.

Stored urine samples were analyzed for aMT6s, 8-OHdG, and MDA; stored nasal fluid samples were analyzed for MDA. Personal exposures to PM_{2.5} and O₃ prior to a health outcome measurement were calculated. Three major analyses were conducted in the Aim 4 study.

First, the associations of personal air pollutant exposures with the indicators of pulmonary physiology were examined. The results show that daily changes in personal exposure to PM_{2.5} were associated with significantly increased small airway resistance, total airway resistance, and airway inflammation (fractional exhaled nitric oxide, FeNO). The findings suggest the importance of reducing personal exposure to PM_{2.5} as part of the asthma management plan to improve airflow limitation.

Second, statistical analyses were conducted to examine the relationships among personal pollutant exposures, nasal fluid MDA, urinary 8-OHdG, urinary MDA, FeNO, and asthma symptom scores. The results showed that increased personal exposures to PM_{2.5} and O₃ exposure were both associated with increased nasal MDA concentrations and worsened asthma symptom scores. Increased nasal MDA concentration was associated with decreased asthma symptom scores indicating worsening of asthma symptoms. These findings support that MDA in the nasal fluid may serve as a useful biomarker for monitoring asthma status, especially in relation to PM_{2.5} and O₃ exposure, two known risk factors of asthma exacerbation.

Third, statistical analyses were conducted to investigate the relationship of urinary aMT6s with personal air pollutant exposures, biomarkers of oxidative stress, and indicators of pulmonary physiology. The results showed that increasing urinary MDA or 8-OHdG concentration and personal exposures to PM_{2.5} and O₃ were associated with increased urinary aMT6s concentrations in asthmatic children. We also found that increased concentration of urinary aMT6s was associated with improved pulmonary inflammation and airway resilience. The results suggest a potential biological mechanism that increased systemic oxidative stress may stimulate the excretion of melatonin as a defense mechanism to alleviate the adverse effects of air pollution exposure.

In summary, the findings from this dissertation research support that endogenously generated melatonin can modulate oxidative, inflammatory, and physiological responses to air pollution exposure in a beneficial way. This dissertation research supports the need for future trials to assess the efficacy or effectiveness of using melatonin supplementation to mitigate the adverse health effects of air pollution exposure at the individual level. This is particularly important for susceptible populations living in highly polluted areas (e.g., developing countries and regions subject to frequent wildfires), people with melatonin deficiency, and those using dirty household fuels.

Dedication

I would like to dedicate this dissertation to my mother, Pin Wang, my grandmother, Jinfeng Sun, my grandfather, Guangrui Wang, and all my family members and friends who have supported me through my education.

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List of Abbreviations

ACME	Average causal mediation effects
ACN	Acetonitrile
ADE	Average direct effects
AFMK	Acetyl-N-formyl-5-methoxykynurenamine
AHU	Air handling unit
aMT6s	6-sulfatoxymelatonin
AUC	Area under the curve
BMI	Body mass index
C-ACT	Childhood Asthma-Control Test
COPD	Chronic obstructive pulmonary diseases
dG	2'-deoxyguanosine
ELISA	Enzyme-linked immunoSorbent assays
ESP	Electrostatic precipitator
FEF ₂₅₋₇₅	The average forced expiratory flow during 25% to 75% of FVC;
FeNO	Fractional exhaled nitric oxide;
FEV ₁	Forced expiratory volume in first second;
FEV ₁ /FVC	The ratio between FEV ₁ and FVC;
FEV ₁ /FEV ₁ predicted	The ratio between FEV ₁ and predicted FEV ₁ ;
FDA	Food and Drug Administration

Fres	Resonant frequency;
FVC	Forced vital capacity;
FVC/FVC predicted	The ratio between FVC and predicted FVC;
GC	Gas chromatography
GSK	GlaxoSmithKline
HEPA	High-efficiency particulate air;
HPLC	High-performance liquid chromatography
IFN- γ	Interferon gamma
IgE	Immunoglobulin E;
IL-1 β	Interleukin 1 β
IL-2	Interleukin 2
IL-4	Interleukin 4
IL-6	Interleukin 6
IL-8	Interleukin 8
IL-10	Interleukin 10
IL-17A	Interleukin 17A
I/O ratio	The ratio between indoor and outdoor concentrations;
IOS	Impulse oscillometry;
IPA	Isopropyl alcohol
IQR	Interquartile range

LC-MS	Liquid chromatography-tandem mass spectrometry
LLOQ	Lower limit of quantification
LMER	Linear mixed-effects regression
LOD	Limit of detection
LOQ	Limit of quantification
MDA	Malondialdehyde
MS	Mass spectrometry
NaOH	Sodium hydroxide
NF- κ B	Nuclear factor kappa B
NLRP3	NLR Family Pyrin Domain Containing 3
NO ₂	Nitrogen dioxide
O ₃	Ozone;
PCOS	Polycystic ovarian syndrome
PEF	Peak expiratory flow
PM _{2.5}	Particles with an aerodynamic diameter $\leq 2.5\mu\text{m}$;
RIA	Radioimmunoassay
ROS	Reactive oxygen species
RSD	Relative standard deviation
R ²	Correlation coefficient
R ₅	Airway resistance measured at 5Hz;

R ₂₀	Airway resistance measured at 20Hz;
R ₅ -R ₂₀	Difference between airway resistance measured at 5Hz and 20Hz;
SES	Socioeconomic status
SPE	Solid phase extraction
SD	Standard deviation
SO ₂	Sulfur dioxide
SVOCs	Semi-volatile organic compounds.
SRM	Selected reaction monitors
TBA	Thiobarbituric acid
TNF- α	Tumor necrosis factor alpha
VOCs	Volatile organic compounds;
WHO	World Health Organization
X ₅	Airway reactance measured at 5Hz;
Z ₅	Airway impedance measured at 5Hz;
8-OHdG	8-hydroxy-2'-deoxyguanosine
¹⁵ N5-8-OHdG	Isotopic 8-OHdG

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Chapter 1. Introduction

1.1 Air pollution health impacts

The data from the World Health Organization (WHO) shows that 91% of the world's population lives in areas where ambient air pollution levels exceed WHO limits in 2020.¹ WHO estimated that exposures to ambient and household air pollution contributed to 4.2 and 3.8 million death per year, respectively.¹

1.1.1 The impacts of air pollution exposure on systemic oxidative stress

Fine particulate matters (PM_{2.5}) and gas pollutants, including ozone (O₃), nitrogen dioxide (NO₂), and sulfur dioxide (SO₂) have been associated with adverse health effects. The underlying mechanisms have been also investigated.² Among them, one major pathophysiologic pathway is via the generation of reactive oxygen species (ROS) in the circulatory system and respiratory tract.³ Some air pollutants, themselves, are ROS, such as O₃, while others can induce ROS by interacting with, epithelial endothelial, and immune cells.⁴ These ROS can further react with critical biomolecules such as DNA and lipids of the cell membranes, which may lead to inflammation and cell death.^{5,6} For example, it was reported that exposure to PM_{2.5} was positively and significantly associated with malondialdehyde (MDA), a stable product of ROS-induced lipid peroxidation.^{7,8} In addition, other studies have reported that increased PM_{2.5} exposure was associated with increased urinary concentration of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a product of DNA oxidative damage.^{9,10}

1.1.2 The impacts of air pollution exposure on inflammation

In addition to oxidative stress, air pollutant exposures can induce airway inflammation by activating inflammatory cytokine/chemokine gene expressions and protein secretion in human bronchial epithelial cells.¹¹ The inflammatory responses may further lead to exacerbation of reduced respiratory function and respiratory symptoms in people with asthma and chronic obstructive pulmonary diseases (COPD).^{12, 13} These inflammatory cytokines and chemokines can further spillover into circulatory system inducing systemic inflammation,¹⁴ which has been accepted as one of the major pathophysiologic mechanisms of air pollution-induced diseases.¹⁵

Many epidemiological studies have investigated inflammatory responses to air pollution exposure in the circulatory and respiratory systems. For example, a study has reported that sub-chronic O₃ exposure was associated with increased levels of proinflammatory cytokines.¹⁶ Other studies reported that exposure to NO₂ for 24 hours was associated with increased levels of interleukin 6 (IL-6), which is a proinflammatory cytokine.¹⁷ In addition, air pollution exposure has been associated with inflammation in the respiratory system. For example, short-term exposure to ambient PM_{2.5} was associated with fraction of exhaled nitric oxide (FeNO, a biomarker of airway inflammation) in COPD patients.¹⁸

1.1.3 The impacts of air pollution exposure on asthma

Asthma is a common chronic and non-communicable disease in both children and adults.¹⁹ The prevalence of asthma is increasing in many regions of the world.²⁰ Asthma is a heterogeneous disease with different phenotypes,²¹ and the causes of asthma are still unclear. Air pollutant exposures have been widely accepted as a major risk factor for asthma exacerbation.²² Increased ambient air pollutant level was associated with increased emergency department visits related to asthma exacerbation.²³ As air pollution levels are elevated in polluted urban atmospheres worldwide,²⁴ it has been an important focus in asthma control.

1.1.3.1 Spirometric lung function

Spirometric lung function is widely used in asthma diagnosis and prognosis. Many epidemiological studies have reported adverse associations between air pollution exposure and pulmonary health conditions in people with asthma. For example, a previous study found that increased 24-hour personal exposure to PM_{2.5} one day prior was associated with decreased peak expiratory flow (PEF) in a group of asthmatic children (n=17) in Seattle, WA.²⁵ Increased personal PM_{2.5} exposure measured in the preceding 24-hours was associated with decreased forced expiratory volume in the first second (FEV₁) in a group of children (n=19) living in San Diego, CA.²⁶ However, other studies have reported nonsignificant associations between PM_{2.5} exposure and lung function.²⁷

1.1.3.2 Airway mechanics

The importance of airway mechanics, measured by impulse oscillometry (IOS), has been increasingly recognized in asthma management. Lung function changes require a persistent stress or relatively strong acute stimulus to the lung. In contrast, airway mechanics are a more sensitive metric used to monitor airway obstruction and airflow limitation. Although clinical benchmark values have not been established, airway mechanics have been increasingly used in asthma management.^{28, 29} However, no study has examined the associations of air pollution exposures and airway mechanics in people with asthma.

1.1.3.3 Airway inflammation

It is widely reported that the pathophysiology of asthma involves airway inflammation.³⁰ As a common asthma treatment medicine, inhaled corticosteroids have been used to suppress airway inflammation.³¹ Among the many biomarkers used in determining airway inflammation, FeNO is the most widely used biomarker of airway inflammation in asthma.³² Many epidemiological studies have investigated the effects of air pollution exposure on airway inflammation in people with asthma. For example, one study reported that personal PM_{2.5} exposure averaged over 2 days prior to the FeNO measurement was associated with a significant increase in FeNO.³³

1.1.3.4 Oxidative stress in the upper respiratory tract

It is well known that air pollution exposure leads to elevated oxidative stress in the lung and the circulatory system. Given that the nose is a prime portal of entry of air pollutants into the human body,³⁴ it is hypothesized that air pollution exposure can also exert oxidative damage in the nose. This oxidative damage may further trigger a mucosal irritating sensation, which exacerbates asthma symptoms. This hypothesis has been tested in cell and animal models finding that air pollutant exposure can induce oxidative stress in the nasal mucosa of rats and human nasal epithelial cells.^{35,36} However, no study has explored the relationships between air pollution exposure and a nasal fluid biomarker of oxidative stress in humans.

1.2 Melatonin

Melatonin is widely known as a hormone excreted by the pineal gland with a marked circadian rhythm.³⁷ It can also be produced by various tissues in the body.³⁸ This is an important hormone that regulates many biological functions, including sleeping, reproduction, and immunity.³⁹

1.2.1 Method for melatonin measurement

Circulating melatonin concentration can be directly measured in the blood. However, it is difficult to accurately measure melatonin levels using a blood specimen for the following reasons. Melatonin is a potent antioxidant and has a short half-life of approximately 1h.⁴⁰ The blood melatonin level can rapidly decrease after blood

collection, because of the short half-life. Moreover, it may be infeasible to collect and timely process blood samples in a non-clinical setting.

Urinary metabolites of melatonin can be a useful surrogate of circulating melatonin level.^{41, 42} Melatonin has many urinary metabolites, including 6-sulfatoxymelatonin (aMT6s), acetyl-N-formyl-5-methoxykynurenamine (AFMK), 6-hydroxymelatonin glucuronide, 6-hydroxymelatonin, and N-acetylserotonin.^{43, 44} Among them, aMT6s has been considered as the major melatonin urinary metabolite under various pathophysiological conditions.⁴¹ In addition, aMT6s is stable in urine for at least two years at -20 °C.⁴² Hence, urinary aMT6s has been widely used as a surrogate of circulating melatonin in many studies.

Some methods have been developed to measure urinary aMT6s using enzyme-linked immunosorbent assays (ELISA), radioimmunoassay (RIA), and high-performance liquid chromatography (HPLC) or gas chromatography (GC) coupled with mass spectrometry (MS).⁴⁵⁻⁵⁰ However, each of these methods has certain shortcomings. For example, ELISA and RIA methods have lower reproducibility due to batch-to-batch variability in the reactivity of antibodies used in the assay. The GC-MS methods involve time- and supplies-consuming derivatization steps yet yielding low recovery.^{51, 52} The existing HPLC-MS method can further improve its limit of detection (LOD).⁴⁷ There is a need to develop a method with improved efficiency, recovery, qualification, and quantification to analyze the concentration of urinary aMT6s.

1.2.2 The effects of melatonin on oxidative stress

Melatonin is a potent antioxidant, and it is effective in reducing oxidative stress via two pathways: direct and indirect pathways.⁵³ First, melatonin can directly scavenge toxic reactive oxygen and nitrogen species.⁵³ Second, melatonin can indirectly reduce oxidative stress by stimulating antioxidant enzymes while suppressing the activity of pro-oxidant enzymes. For example, melatonin was found to stimulate antioxidative enzymes including glutathione peroxidase and glutathione reductase.⁵⁴ Furthermore, melatonin upregulates the synthesis of glutathione, a highly effective antioxidant, and synergizes with classic free radical scavengers to improve the reductive potential of tissues and fluids.⁵⁵ Melatonin can also reduce oxidative damage by binding to heavy metals, including iron, zinc, lead, copper, and cadmium, which can generate ROS through Fenton reaction.⁵⁶ These indirect antioxidant functions of melatonin further leverage this molecule as being a key endogenous factor in limiting oxidative damage.

1.2.3 The effects of melatonin on inflammation

Melatonin is an immune modulator with both anti- and pro-inflammatory properties.⁵⁷ As a widespread anti-inflammatory molecule, melatonin can modulate the inflammasome (NLRP3), which responds to various inflammation-related molecular signals.⁵⁸ The activation of NLRP3 is protective against invading pathogens; however, its excessive activation can lead to tissue injury.⁵⁹ In addition, melatonin can modulate the nuclear factor kappa B (NF- κ B) signaling pathway, which plays an important role in

regulating many important genes involved in cellular inflammatory responses.^{58, 60}

Melatonin could also reduce inflammation by inhibiting neuronal nitrogen oxide synthases and downregulating cyclooxygenase-2 activities.⁶¹

On the other hand, the pro-inflammatory actions of melatonin were mainly found on immune cells. For example, it has been reported that, in response to melatonin, monocytes, monocyte-derived cells, and T-helper cells would release increased level of pro-inflammatory cytokines, including interleukin-2 (IL-2), interleukin-5 (IL-5), Interferon-gamma (IFN- γ), and Tumor necrosis factor-alpha (TNF- α).⁶² However, it is still unclear whether these pro-inflammatory effects of melatonin also exist in the human body.

To date, the general findings indicate that melatonin enhances the defense systems of organisms.⁶² Taking melatonin supplementation has been previously reported to suppress inflammatory responses in both animal models and humans. For example, a randomized trial reported that oral administration of 25 mg/d melatonin for 6 months was associated with significantly decreased blood concentration of pro-inflammatory cytokines in mice, including interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and TNF- α .⁶³ Another study found that receiving daytime administration of melatonin (100 mg) before endotoxemia reduced IL-1 β level, a pro-inflammatory cytokine, in humans.⁶⁴ However, some autoimmune diseases, such as arthritis, may not be benefited

from melatonin supplementation, concerning melatonin may worsen the disease condition by enhancing immune responses.⁶²

1.2.4 The effects of melatonin on asthma

The effects of melatonin on asthma are poorly understood. Some studies have suggested a therapeutic potential of melatonin in asthma treatment.⁶⁵ For example, melatonin can suppress oxidative damage and modulate inflammatory responses, which are related to asthma exacerbation.⁶⁵ In addition, melatonin can inhibit eosinophil peroxidase, which plays an important role in producing ROS and asthma pathogenesis.⁶⁶ Another study reported that melatonin can inhibit mucus production by suppressing related gene expression, which helps to improve asthma symptoms.⁶⁷

On the other hand, some epidemiological studies have reported adverse health effects of melatonin on asthma. For example, one study found that peak melatonin levels in a group of asthmatic patients were significantly higher than that of healthy controls.⁶⁸ The authors further reported a significant and adverse association of circulating melatonin levels and lung function in people with asthma.⁶⁸ However, the underlying biological mechanisms are still unclear. Based on the results of this previous study, melatonin supplementation was not recommended as a sleep promoter for asthmatic patients.⁶⁵ Further studies are needed to examine the health effects of melatonin on asthma before it can be routinely recommended or discouraged in asthma control.

1.3 Specific aims

The primary goal of this dissertation is to examine the role of melatonin in pathophysiological responses to air pollution exposure. As reported in many previous studies, air pollution exposure can increase levels of systemic oxidative stress and inflammation and worsen asthma symptoms. In addition, melatonin can modulate systemic oxidative stress and inflammatory responses and may also affect the pulmonary physiology in people with asthma. However, it is still unknown how melatonin may affect the relationships of air pollution exposure with systemic oxidative stress, inflammation, and pulmonary physiology in people with asthma. The goal of this dissertation research is to fill in the knowledge gap concerning whether and how melatonin, at the natural level (i.e., without melatonin supplementation), affects the relationships of air pollution exposure with biomarkers of oxidative stress and inflammation in adults as well as with lung function, airway mechanics, and oxidative stress in asthmatic children. The goal is achieved through the following four aims.

1.3.1 Aim 1: Develop an analytical chemistry method to simultaneously measure urinary 6-sulfatoxymelatonin (aMT6s) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) using liquid chromatography-tandem mass spectrometry (LC-MS) (Chapter 2).

Aim 1 of this dissertation is to develop a novel analytical method using LC-MS technique to simultaneously measure urinary aMT6s and 8-OHdG. As a stable product of DNA oxidative damage, 8-OHdG can be measured in urine and is a biomarker of systemic oxidative stress. The existing HPLC-based methods for 8-OHdG analysis

require a solid phase extraction (SPE) pretreatment procedure, which is supplies- and time-consuming.^{69, 70} The new method aims to reduce the cost of the analytical method by replacing the SPE with a liquid-liquid extraction to save both time and supplies. The cost-saving is further achieved by making the method capable of quantifying both 8-OHdG and aMT6s simultaneously.

1.3.2 Aim 2: Investigate the role of melatonin in oxidative stress responses to air pollution exposure in healthy adults (Chapter 3).

Aim 2 of this dissertation is to investigate the role of melatonin in oxidative stress responses to air pollution exposure in healthy adults. Urine samples and relevant exposure data have been obtained from two previous studies, which originally investigated the cardiopulmonary health effects of air pollution exposure.^{14, 71} In Aim 2, urinary aMT6s and 8-OHdG were further measured using the method developed in Aim 1.

The relationships among short-term air pollution exposure, urinary aMT6s, and two biomarkers of oxidative stress (urinary MDA and 8-OHdG) were examined. The potential confounding, mediation, and modification effects of urinary aMT6s on the relationships of air pollution exposure with MDA and 8-OHdG were investigated. This was the first study to investigate the role of urinary aMT6s in the relationships of air pollution exposure and biomarkers of systemic oxidative stress.

1.3.3 Aim 3: Investigate the role of melatonin in inflammatory responses to air pollution exposure in healthy adults (Chapter 4).

Aim 3 of this dissertation is to investigate the role of melatonin in inflammatory responses to air pollution exposure in healthy adults. Urine and blood samples, urinary MDA concentration, and relevant exposure data were obtained from a previous study of the cardiopulmonary health effects of air pollution exposure.¹⁴ Aim 3 is presented in the Chapter 4 including Part A and Part B.

In Part A of Chapter 4, 9 inflammatory cytokines were measured in the blood samples. Personal air pollutant exposures were associated with these cytokines and urinary MDA. In Part B of Chapter 4, urinary aMT6s was measured and associated with both air pollution exposure and the 9 inflammatory cytokines. In addition, the mediation effects of urinary aMT6s on the relationships of air pollution exposure and inflammatory cytokines were investigated in Part B. This was the first study to explore the role of melatonin in inflammatory responses to air pollution exposure.

1.3.4 Aim 4: Investigate the role of melatonin in physiological and oxidative stress responses to air pollution exposure in asthmatic children (Chapter 5)

Aim 4 of this dissertation is to investigate the role of melatonin in physiological and oxidative stress responses to air pollution exposure in asthmatic children. The urine and nasal fluid samples and pulmonary physiology outcomes were obtained from a previous panel study that investigated the pulmonary health effects of air purification intervention.⁷² Pulmonary physiology outcomes included airway mechanics, lung

function, airway inflammation, and asthma symptom scores. Aim 4 is presented in the Chapter 5 including Part A, Part B, and Part C.

In Part A, 24 h average personal PM_{2.5} exposure and daily maximum 8 h average personal O₃ exposure 0 to 6 days (lag day 0–6) prior to biospecimen collection were calculated. The associations of personal air pollutant exposures with indicators of pulmonary physiology, including airway mechanics, lung function, airway inflammation were examined.

In Part B, MDA in the nasal fluid samples was measured. The associations of air pollution exposure with nasal MDA and asthma symptom scores were examined. In addition, the relationships of nasal MDA and asthma symptom scores were investigated to explore whether nasal MDA is useful in assisting with asthma status monitoring.

In Part C, urinary aMT6s, MDA, and 8-OHdG were further measured. The associations of urinary aMT6s concentration with personal air pollutant exposures, systemic oxidative stress, airway mechanics, lung function, and airway inflammation were examined. These associations were investigated to understand how air pollution exposure affect endogenous melatonin level, and how endogenous melatonin affects respiratory health outcomes.

Chapter 2. Development of an analytical chemistry method to simultaneously measure urinary 6-sulfatoxymelatonin (aMT6s) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) using liquid chromatography-tandem mass spectrometry (LC-MS)

This chapter addresses Aim 1 of this dissertation research. It is adapted with permission from He, L.; Liu, X.L.; Zhang, J.J. Simultaneous quantification of urinary 6-sulfatoxymelatonin and 8-hydroxy-2'-deoxyguanosine using liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B*. 2018, 1095, 119-126 (Publisher: Elsevier). The accompanying supporting information to the published manuscript is included in Appendix A. I led the method development and manuscript writing. Dr. X. Liu assisted with experimental design and procedures. Dr. J. Zhang advised on the study design, data interpretation, and manuscript writing.

2.1 Introduction

Reactive oxygen species (ROS), such as free radicals, are recognized for playing a dual role in human body.⁷³ A low or moderate level of ROS could be beneficial to various physiological processes, while overproduction of ROS results in increased body burden of oxidative stress that leads to damages to membrane lipids, proteins, and DNA, all are vital biomolecules supporting the normal function of the human body.^{73, 74} DNA mutation due to oxidative stress has been strongly associated with cellular aging, cancer, and degenerative diseases.⁷⁵ Particularly, ROS attacks 2'-deoxyguanosine (dG) in the DNA strand forming 8-OHdG (Figure 1).⁷⁶ During repairing process, 8-OHdG within

the DNA strand is repaired and released from cells to blood and then later excreted in urine.⁷⁷⁻⁸¹ The excreted 8-OHdG remains stable in urine and hence has been commonly used as a biomarker of oxidative stress.^{82, 83}

Melatonin is an hormone excreted by pineal gland of animals and it's widely known to be important in regulating circadian rhythms.⁸⁴ It is also a potent ROS scavenger and plays a key role in DNA damage repairing and hence may affect urinary excretion of 8-OHdG.⁸⁵⁻⁸⁹ A recent study found that both urinary 8-OHdG and circulating melatonin concentrations were lower in night shift workers than in day shift workers.⁸⁷ In this case, a lower urinary 8-OHdG level might not necessarily indicate a lower level of oxidative stress, as lower melatonin levels in the night-shift workers may have decreased the repair of the oxidized DNA.⁹⁰⁻⁹³ Therefore, only reporting 8-OHdG level, as commonly done in previous studies, may not accurately reflect the initial ROS concentrations (oxidative stress). It is important to consider or adjust melatonin levels when measuring 8-OHdG for monitoring of oxidative stress.

Several melatonin metabolites in urine have been reported, including 6-sulfatoxymelatonin (aMT6s, Figure 1), 6-hydroxymelatonin glucuronide, 6-hydroxymelatonin, acetyl-N-formyl-5-methoxykynurenamine (AFMK), and N-acetylserotonin.^{43, 44} Among these metabolites, aMT6s has been considered as the main melatonin metabolite excreted in urine under various pathophysiological conditions, and there is a strong correlation between urinary aMT6s and plasma melatonin

concentrations.^{41, 42} Thus, the measurement of urinary aMT6s is a noninvasive and convenient way of monitoring melatonin in the body.

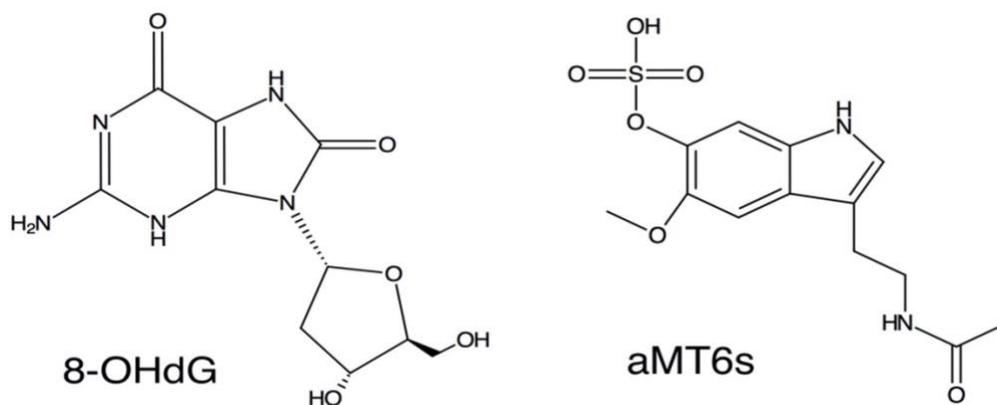


Figure 1. Structure of aMT6s and 8-OHdG.

In this study, we aim to develop a novel method to simultaneously measure urinary 8-OHdG and aMT6s. Published analytical methods for urinary 8-OHdG and those for urinary aMT6s individually include enzyme-linked immunosorbent assays (ELISA), radioimmunoassay (RIA), and gas chromatography (GC) or high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) or other detectors.⁴⁵⁻⁵⁰ In general, ELISA methods have lower reproducibility due to batch-to-batch variability in reactivity of antibodies used in the assay. The existing HPLC-based methods for 8-OHdG analysis require a multi-step solid phase extraction (SPE) procedure that is time and supplies consuming.^{69, 70} Over the last decade, few efforts have been made to improve the analytical method for 8-OHdG. Existing methods for aMT6s analysis using a GC-MS technique involve time and supplies consuming derivatization processes yet yielding low recovery.^{51, 52} Recently a high-performance liquid chromatography-tandem

mass spectrometry (HPLC-MS/MS) based method has been developed for aMT6s, eliminating the need for the derivatization step used in the GC-MS methods. However, the limit of detection (LOD) of this method can still be improved.⁴⁷ Compared to the existing methods, the new HPLC-MS/MS method reported in this study has streamlined sample pretreatment processes and optimized spectrometric parameters to achieve improved efficiency, recovery, qualification, and quantification. Finally, the method was successfully applied to measure first morning and midday urine voids for both aMT6s and 8-OHdG from five volunteers, showing concentrations for both compounds all above limits of quantification and within expected ranges.

2.2 Experimental

2.2.1 Chemicals and reagents

High-purity ($\geq 98\%$) aMT6s was obtained from Santa Cruz Biotechnology (Dallas, TX, USA) and 8-OHdG ($\geq 98\%$) was from Sigma-Aldrich (St Louis, MO, USA). ¹⁵N5-8-hydroxy-2'-deoxyguanosine (¹⁵N5-8-OHdG) (98%), used as the internal standard was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Methanol, acetonitrile (ACN), and water were purchased from Fisher Chemical (Fair Lawn, NJ, USA); isopropyl alcohol (IPA) was from Honeywell (Muskegon, MI, USA); and ammonium acetate was from Fisher Scientific (Ottawa, ON, Canada). All these reagents were HPLC-MS grade. Sodium hydroxide was from Sigma-Aldrich (St. Louis, MO, USA).

2.2.2 Instrumentation

All qualification and quantifications of the analytes were carried out using an HPLC-MS/MS system including an Accela Open AS HPLC, an Accela 1250 pump, a TSQ Quantum mass spectrometer, and a Xcalibur 2.0 data system software (Thermo Fisher Scientific: San Jose, CA, USA). The separation of compounds was achieved using a Synergi™ 4µm Hydro-RP 80 Å, LC column 50 x 1 mm (Phenomenex, Torrance, CA, USA).

2.2.3 Mass spectrometry and chromatography parameters optimization

The ESI ion source in the negative ion mode has been used in previous methods for aMT6s, whereas both the negative and positive ion modes have been used for 8-OHdG analysis.^{94, 95} Thus, the ESI negative mode was chosen to simultaneously measure both compounds in this new method. Different collision energies were tested to achieve the highest sensitivity. The temperature of the auto-sampler was set at 25°C and the injection volume was 20 µL. Two selected reaction monitors (SRM) were used to quantify and identify 8-OHdG and aMT6s, respectively. Final key parameters of the mass spectrometry upon optimization are shown in Table 1. The mobile phase included 5mM ammonium acetate as solvent A and methanol as solvent B. The chromatographic gradient program used is shown in Table 2.

Table 1. Parameters used for the HPLC-MS/MS analysis of 8-OHdG and aMT6s.

	8-OHdG	aMT6s
Spray voltage (V)	4,000	4,000
Vaporizer temperature (°C)	300	300
Sheath gas pressure (arb)	35	35
Aux gas pressure (arb)	10	10
Capillary temperature (°C)	350	350
Tube lens offset (V)	-67	-67
Ionization mode	Negative	Negative
Normalized collision energy	25	20
Time range (min)	3-4	3-4
SRM transition (m/z)	282.1-192.1 (Quantification) 282.1-166.0 (Identification, 30 V)	327.1- 247.1 (Quantification) 327.1-58.0 (Identification,
Scan width (m/z)	0.1	0.1
Scan time (s)	0.1	0.1
Q1 (FWHM)	0.7	0.7

Table 2. HPLC mobile phase gradient program used to separate 8-OHdG and aMT6s in urine extracts.

Time (min)	Solvent A	Solvent B	Flow rate (µL/min)
0	95%	5%	200
0.5	95%	5%	200
4	0	100%	200
5	0	100%	200
6	95%	5%	200
8	95%	5%	200

2.2.4 Standard solution preparation

The stock individual standard solutions of 8-OHdG and aMT6s at concentration of 1mg/mL were prepared by dissolving 1 mg of each compound into 1 mL of dimethyl sulfoxide (DMSO). The stock solutions for 8-OHdG and those for aMT6s were diluted with water to obtain a series of concentrations for each analyte, respectively. Then by mixing the two solutions using equal amounts, standard solutions containing both analytes with known concentrations were created for making calibrations curves. A series of 7 solutions with a concentration range of 0.1-100 ng/mL for both 8-OHdG and aMT6s were used.

2.2.5 Sample preparation

The urine samples tested in this study were obtained from five anonymous volunteers. Each volunteer provided a first morning urine void and a midday urine void on a same day, and urine samples were analyzed within the same day of collection. These samples were collected under a study protocol that had been approved by the Ethics Committee of Shanghai First People's Hospital (2015-38) and had also been agreed upon by the Duke Kunshan University Campus IRB. Sample pretreatment started with centrifugation of urine at 1,150 relative centrifugal force for 10 minutes using a centrifugal filter unit (Durapore PVDF 0.22 um, Merck Millipore, Cork, Ireland). Then a 397.5 μ L of supernatant was mixed with 10 μ L internal standard (50 ng/mL ¹⁵N5-8-OHdG), 2.5 μ L 1M sodium hydroxide (NaOH), and 90 μ L methanol. After mixing

well, the mixture was filtered while centrifuging for 3 min at 1,150 relative centrifugal force. Finally, the resulting mixture after filtration was used for HPLC-MS/MS analysis. Experiments were conducted using various types and proportions (20%, 40%, 60%, 80%) of organic solvents (IPA, ACN, methanol) and a range of pH values (pH=5-12) to find optimal extraction conditions. Urinary creatinine was measured using a widely accepted spectrophotometric method (G 10s UV-VIS, Thermo Fisher Scientific, Madison, WI, USA) to address urine dilution factor.⁹⁶

2.2.6 Validation

The method performance was validated according to the guidelines for bio-analytical method recommended by U.S. Food and Drug Administration (FDA) using indicators for linear range, correlation coefficient (R^2), limit of detection, limit of quantification (LOQ), lower limit of quantification (LLOQ), retention time standard deviation (SD), inter-day & intra-day variability expressed as RSD, and recovery.⁹⁷

Three calibration curves were constructed on each day for three separate days to estimate R^2 , linear range, LOD, and LOQ. LOD and LOQ were calculated as 3 and 10 times the signal to noise ratio, respectively. LLOQ was determined as the lowest concentration of analyte that could give a peak with an accuracy of 80-120% and precision of 20%.⁹⁸

To determine variability in retention time, we calculated SD of 27 retention time values determined as follows: At each of Day 1, 2, and 3, we had 3 replicate runs at each

of the three standard concentrations (10ng/mL, 50ng/mL, and 100ng/mL) prepared in DI water.

To determine method reproducibility, we conducted two sets of experiments. The first set was conducted using three standard concentrations in water (1ng/mL, 10ng/mL, and 100ng/mL). Each standard solution was separated into 5 aliquots, each of which was analyzed on Days 1, 2, and 3, respectively. Intra-day variability for each concentration was defined as RSD from the Day 1; and inter-day variability for each concentration was determined as the average of intra-day variability (RSD) of Days 1,2, and 3. The second set of experiment was conducted to evaluate method reproducibility in the urine matrix. A series of 347.5 μ L aliquots of a midday urine void were mixed with 10 μ L internal standard, 2.5 μ L 1M NaOH, 90 μ L methanol, and spiked with 50 μ L of a standard mixture containing 250, 500, and 1000 ng/mL aMT6s and 8-OHdG, respectively. All experiments were conducted in triplicates. The variability was expressed as relative standard deviation (RSD) from the triplicates at each concentration, as RSD is expected to depend on analyte concentrations.

To determine recovery and matrix effects, we added the same amount of the standard mixture with different concentrations into a urine matrix and a deionized water sample to constitute low (10 ng/mL), middle (50 ng/mL), and high (100 ng/mL) concentrations, respectively. The standard mixture contained isotopic 8-OHdG ($^{15}\text{N}5\text{-}8\text{-OHdG}$). We made the water matrix and urine matrix each to contain 50 ng/mL $^{15}\text{N}5\text{-}8\text{-OHdG}$.

OHdG as natural urine contains negligible amount of this isotopic 8-OHdG. However, since we could not obtain isotopic aMT6s, the urine matrix had slightly higher aMT6s concentration than the water matrix. The urine matrix underwent the entire pretreatment procedures before analysis, as described above, while the water matrix was directly analyzed with the HPLC-MS/MS system. The ratios of respective results (area under the curve, AUC) were calculated. The recovery was calculated using the formulas shown below.

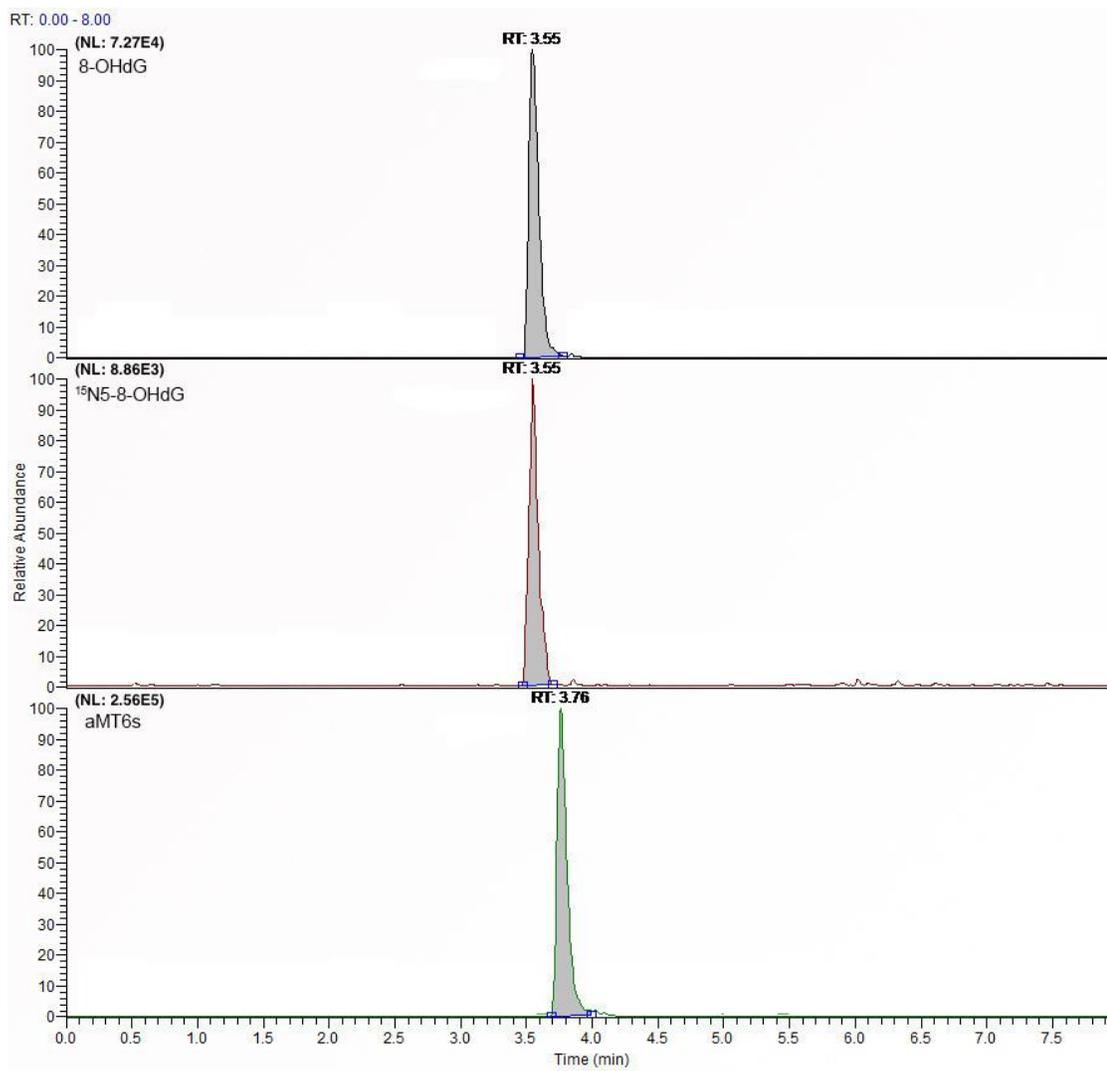
$$Recovery_{8-OHdG}(\%) = \frac{AUC_{8-OHdG}/AUC_{^{15}N5-8-OHdG}(Urine\ matrix)}{AUC_{8-OHdG}/AUC_{^{15}N5-8-OHdG}(Water\ matrix)}$$

$$Recovery_{aMT6s}(\%) = \frac{AUC_{aMT6s}(Urine\ matrix)}{AUC_{aMT6s}(Water\ matrix)}$$

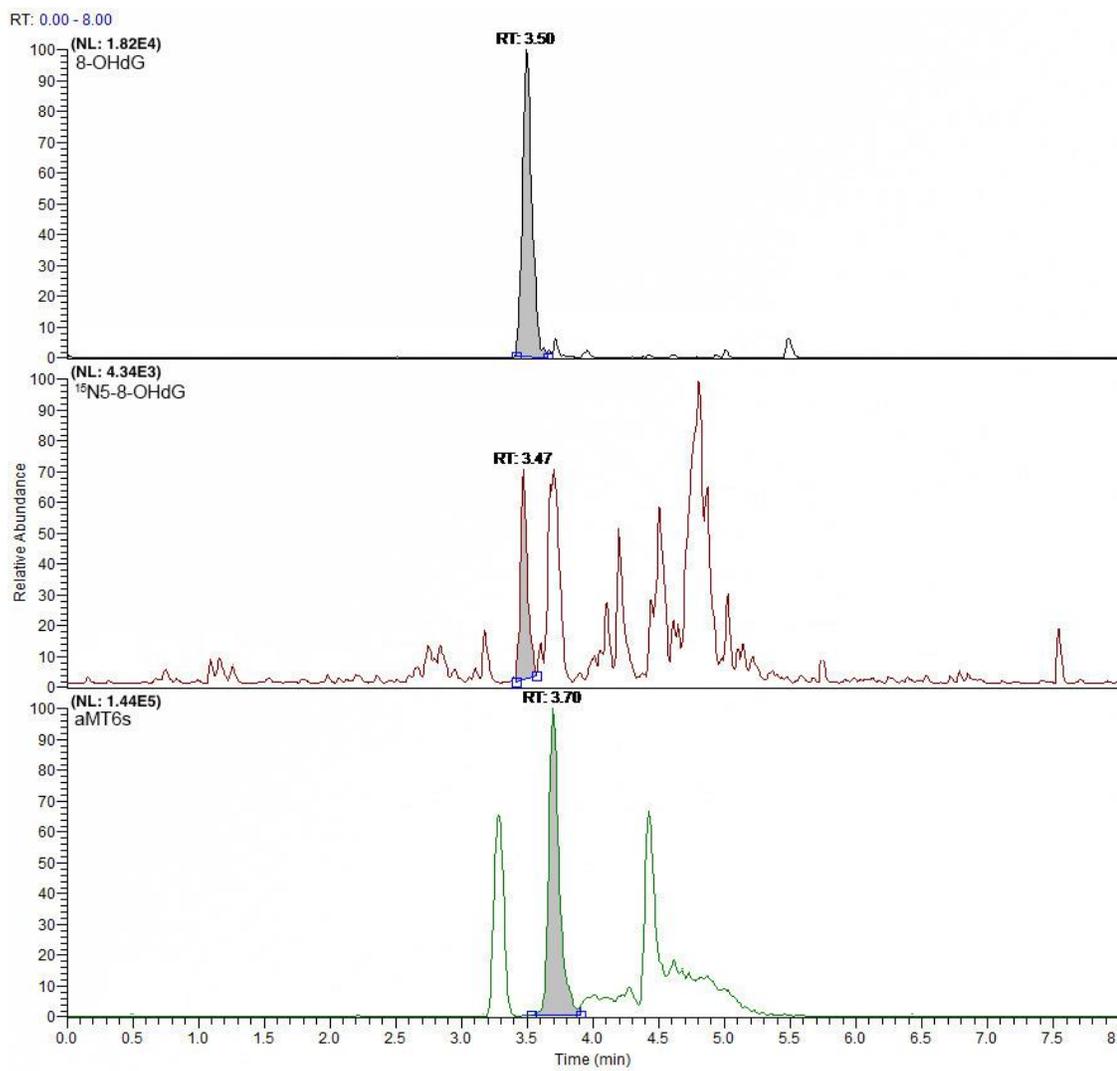
2.3 Results and discussion

2.3.1 HPLC-MS/MS analysis

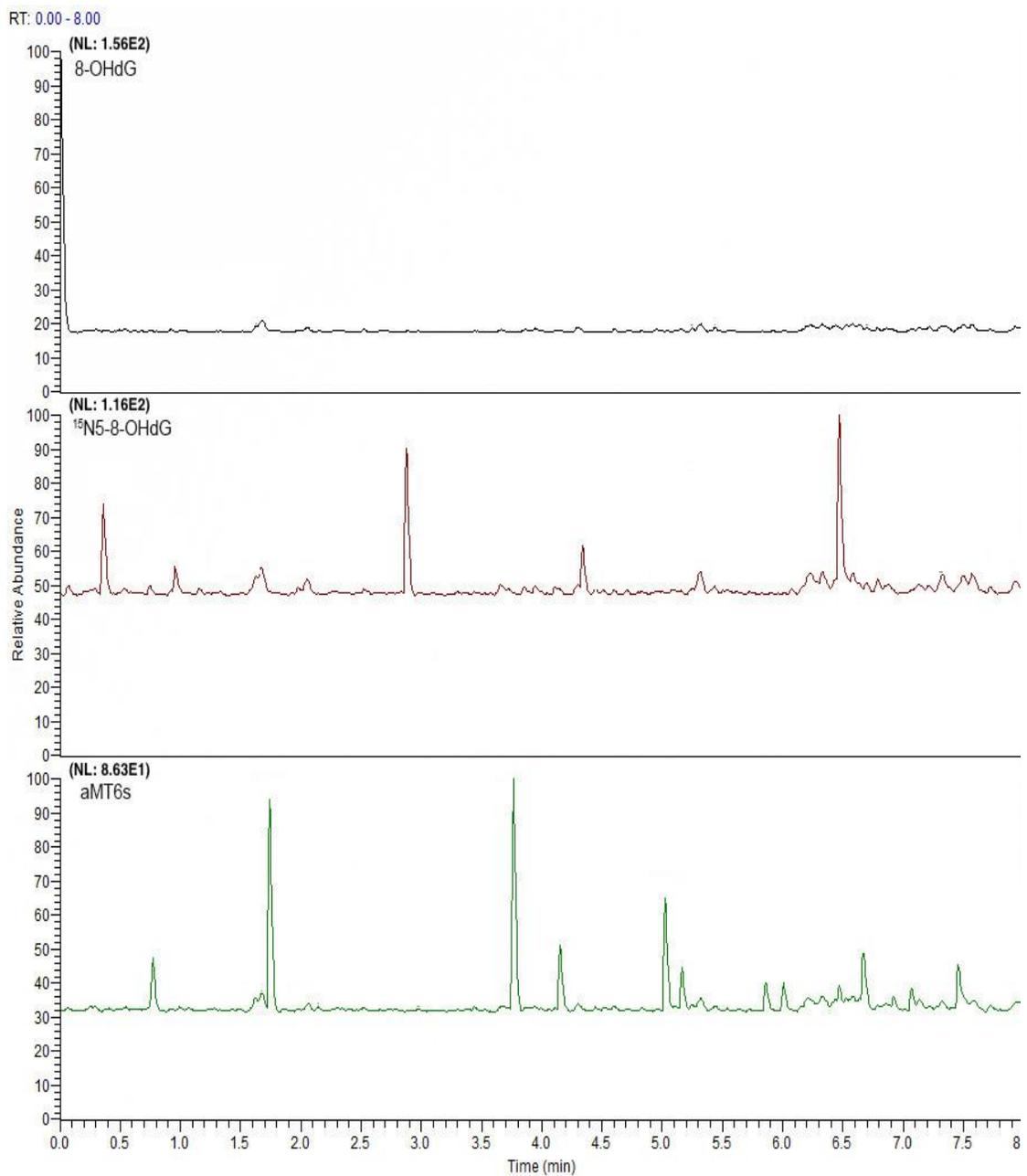
The HPLC-MS/MS chromatograms of aMT6s, 8-OHdG, and ¹⁵N5-8-OHdG showed a complete separation in both water and urine matrix (Figure. 2). The retention times were 3.73±0.03 min, 3.52±0.03 min, and 3.52±0.03 min for aMT6s, 8-OHdG, and ¹⁵N5-8-OHdG, respectively. Based on the published data, the fragmentation transition to quantify 8-OHdG (m/z) was 282.1 to 192.1,⁹⁴ aMT6s (m/z) was 327.1 to 247.1,⁹⁵ and ¹⁵N5-8-OHdG (m/z) was 287.1 to 197.1 & 171.1.⁹⁴ The fragmentation transition to identify 8-OHdG (m/z) was 282.1 to 166.0, and aMT6s was 327.1 to 58.0.



a



b



c

Figure 2. Chromatograms of LC-MS/MS determination of 8-OHdG, ¹⁵N5-8-OHdG, and aMT6s in water with spiked (a), urine with spiked (b), and water without spiked (c).

2.3.2 Sample pretreatment conditions

The sample pretreatment method was optimized by adjusting the type and proportion of organic solvents and the pH of urine sample. Among methanol, acetonitrile, and isopropyl alcohol, methanol showed the best extraction performance. Four different concentrations of methanol (20%, 40%, 60%, 80%) were then tested; and results showed that 20% of methanol resulted in the best performance. Among a range of pH values tested, making the pH of a urine solution around 7, resulting from adding 0.5% of 1M NaOH, generated the optimal extraction performance for both aMT6s and 8-OHdG. The detailed extraction conditions are shown in the supplemental information (Table A1, A2, A3).

Compared with the conventional solid phase extraction (SPE), this newly developed sample pretreatment process using methanol for extraction and pH adjustment substantially reduced the extraction time and the urine volume required and eliminated the use of costly SPE cartridges for analyzing 8-OHdG.^{48,99} As reported in previous studies, aMT6s has been mainly analyzed with ELISA and RIA methods. The current method reduced the time for analyzing aMT6s compared with that using ELISA and RIA, which usually take several hours for incubation. This new method is able to analyze the two compounds in a single HPLC run.

2.3.3 Method performance

Table 3. Method performance for simultaneous measurements of aMT6s and 8-OHdG.

Standards	Linear Range (ng/mL)	Correlation coefficient (R ²)	Retention times SD (min)	LOD (ng/mL)	LOQ (ng/mL)	LLOQ (ng/mL)	Recovery (SD) (%)
6-sulphatoxymelatonin (aMT6s)	0.5-100	0.9999	0.03	0.1	0.3	0.3	88 (4)
8-hydroxy-2'-deoxyguanosine (8-OHdG)	1.5-100	0.9985	0.03	0.5	1.5	1.0	102 (2)

Table 4. Intra-day and inter-day variability for aMT6s and 8-OHdG.

	Concentration	Intra-day variability (RSD) (%)	Inter-day variability (RSD) (%)
aMT6s	1 ng/mL	10.0	12.5
	10 ng/mL	4.5	4.8
	100 ng/mL	6.5	5.9
8-OHdG	1 ng/mL	10.3	15.8
	10 ng/mL	7.7	7.4
	100 ng/mL	5.5	5.1

The method performance is shown in Table 3 and 4. The method shows a good linearity ($R^2 > 0.9999$ for aMT6s and R^2 around 0.999 for 8-OHdG) in the linear range. The retention time for both targets was stable with a SD around 0.03 min (0.8% RSD) (n=27). The intra-day and inter-day variability (RSD) ranged from 4.5% to 12.5% and 5.1% to 15.8% for aMT6s and 8-OHdG, respectively, decreasing as analyte concentration increased. Similar reproducibility was obtained for both the analytes using standards spiked into a human urine sample (see Table A4), indicating that the urine matrix did not affect the reproducibility of the method. The intra-day and inter-day variability for

8-OHdG and aMT6s in this new method, shown in Table 4, are in the range of those reported in previous studies. For example, a previous HPLC-MS-MS 8-OHdG method reported intra-day and inter-day RSD values of 4.8%-8.4% and 2.4%-18.8%, respectively.¹⁰⁰ A previous HPLC-MS method for aMT6s had intra-day and inter-day RSD values of 0.02%-20.83% and 0.18%-22.5%, respectively.⁴⁷

The recovery for 8-OHdG and that for aMT6s were 102% (SD=2%) and 88% (SD=4%), respectively. Isotopic ¹⁵N5-8-OHdG is a well-established internal standard for 8-OHdG analysis. However, no internal standard was applied to the aMT6s analysis for two reasons. The first was that there had not been a well-established internal standard (such as isotope labeled) for aMT6s; and the other was that the current method, without the use of an aMT6s internal standard, already resulted in a well-resolved peak for aMT6s, a high recovery (90%), and a small inter-day and intra-day variability (see Table 4). However, because no internal standard was applied, the aMT6s analysis may be considered as being semi-quantified in this new method. The recovery of 8-OHdG in this new method falls within those previously reported (96.4%-111.2%).⁷⁵ There is a substantial improvement in recovery of aMT6s in this new method compared with that of previous GC-MS method reporting a 11.7% recovery.⁴⁶

LOD, LOQ, and LLOQ were determined for both compounds (Table 3).

According to the existing literature, 8-OHdG can be analyzed under either the ESI positive ion or the negative ion mode, and the instrument response is generally higher

under the positive mode than under the negative ion mode.⁹⁴ The LOD in this new method using the negative ion mode was 0.5 ng/mL, which was about 25 times higher than LODs reported in a previous HPLC-MS/MS method using the positive ion mode (0.02 ng/mL).¹⁰¹ However, this new method has the sensitivity 10 times higher than the HPLC-ECD method with a LOD of 5 ng/mL, which is still adequate for detecting 8-OHdG concentrations in human urine samples.^{102, 103} The LOD for aMT6s in this new method is 2 times, 8 times, and 2.5 times lower than those reported in previous HPLC-MS/MS (0.22 ng/mL), ELISA (0.8 ng/mL), and radioimmunoassay (0.25 ng/mL) methods, respectively.^{47, 104, 105}

2.3.4. Urine sample validation

Urine samples collected from five adult volunteers were analyzed using the new method to demonstrate its applicability. All samples measured had aMT6s and 8-OHdG concentrations above the LLOQ reported in this method of 0.3 ng/mL and 1ng/mL, respectively, and within the linear range of 0.5-100 ng/mL and 1.5-100 ng/mL, respectively. Results show a significant ($p = 0.02$ by paired t test) and substantial (by 2.6-fold) difference in aMT6s concentration between the first morning urine voids and midday (around noontime) urine voids (Figure 3). It is known that melatonin is mainly excreted during the nighttime, which is consistent with our findings in this study.¹⁰⁶ The creatinine adjusted concentrations of aMT6s measured in the 5 volunteers fall within the concentrations reported in the literature. The mean and range of aMT6s in first morning

urine voids measured in this study were 26.6 ng/mg and 16.44-41.8 ng/mg, comparable to the range of 6.9-110.8 ng/mg reported for adults in the literature.¹⁰⁷ The mean and range of aMT6s in daytime urine voids were 10.0 ng/mg and 3.5-20.9 ng/mg measured in this study, which is also consistent with previous studies reporting that aMT6s level in first morning urine voids were about 2-5 fold larger than that in daytime urine voids.¹⁰⁸

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The creatinine adjusted mean and range of 8-OHdG measured in all urine voids of the 5 volunteers were 9.2 ng/mg and 1.9-28.6 ng/mg, comparable to the range of 1.8-86.0 ng/mg reported for adults in the literature.¹¹⁰ No significant difference ($p=0.83$ by paired t test) in 8-OHdG concentration was observed between first morning and midday urine voids. This is consistent with the existing understanding that the urinary excretion of 8-OHdG does not have a clear diurnal pattern.¹¹¹

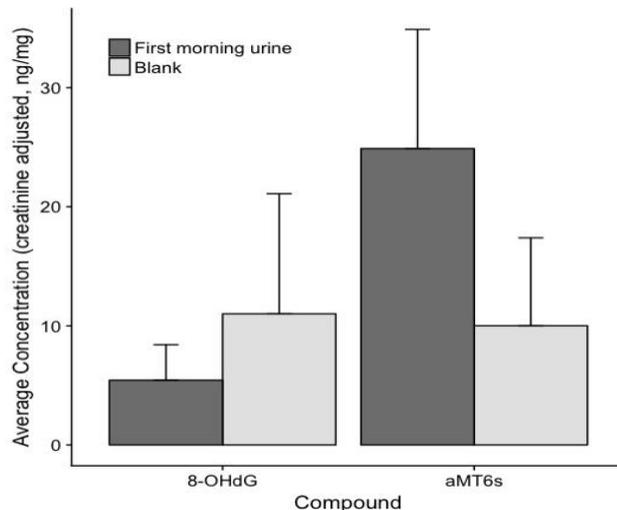


Figure 3. The average concentrations of aMT6s and 8-OHdG between first morning urine and midday urine (blank) from five adults.

2.4 Conclusion

A cost-effective analytical method has been developed to simultaneously measure 8-OHdG and aMT6s in human urines. The method, by replacing a multi-step solid phase extraction step with a liquid-liquid extraction procedure, reduced sample treatment time and cost. Using an HPLC-MS/MS system under the negative ion mode, this new method is capable of measuring human urine samples with adequate precision, accuracy, and sensitivity for both 8-OHdG and aMT6s. Given that urinary 8-OHdG is a widely used biomarker of oxidative stress and that melatonin (the parent compound of aMT6s) could modify urinary excretion of 8-OHdG, it is useful to measure these two compounds together in future environmental health and biomedical studies involving oxidative stress related pathophysiological pathways.

Chapter 3. The role of melatonin in oxidative responses to air pollution exposure in healthy adults.

This chapter addresses Aim 2 and is adapted with permission from He, L.; Cui, X.; Xia, Q.; Li, F.; Mo, J.; Gong, J.; Zhang, Y.; Zhang, J. J., Effects of personal air pollutant exposure on oxidative stress: Potential confounding by natural variation in melatonin levels. *International journal of hygiene and environmental health*. 2020, 223, (1), 116-123 (Publisher: Elsevier). The accompanying supporting information is included in Appendix B. In this Aim study and the published manuscript, I analyzed the urine samples for aMT6s, 8-OHdG, and MDA, conducted the statistical analysis, and led the writing and revising of the manuscript. The coauthors contributed to the study design (JZ), manuscript revisions (JZ and XC), sample and data collection in the original study (JZ, YZ, XC, FL, JG, JM), sample analysis (XC and QX), and discussions (JZ and XC).

3.1 Introduction

It is widely accepted that one of the pathophysiologic pathways linking air pollutant exposure and adverse health effects is via the generation of reactive oxygen species (ROS) in the respiratory tract and the circulatory system. ROS can react with critical biomolecules such as DNA and lipids of the cell membranes^{5,6}. Stable products of these ROS reactions excreted in biological fluids can be used as biomarkers of oxidative stress.^{112, 113} For example, malondialdehyde (MDA) is a stable product of ROS-induced lipid peroxidation; and 8-hydroxy-2'-deoxyguanosine (8-OHdG) is formed within a DNA strand as the product of 2'-deoxyguanosine (dG) oxidation by ROS.^{112, 114}

During DNA repairing process, 8-OHdG within the DNA strand is repaired and released from cells and subsequently excreted into the urine.¹¹⁴

Previous epidemiologic studies have examined associations between air pollutant exposure and urinary concentrations of MDA and 8-OHdG, generating somewhat inconsistent findings. Some studies found a positive association of PM_{2.5} (particles with a diameter $\leq 2.5 \mu\text{m}$) with MDA,^{7, 8} while some found either a negative¹⁴ or non-significant association.^{115, 116} Similarly, positive,^{9, 10} negative, or non-significant^{116, 117} associations have been reported between urinary 8-OHdG and PM_{2.5} exposure. The inconsistency has been attributed to differences in study design, statistical power, subject demographic characteristics, exposure assessment inaccuracy, and potential confounders. Here we hypothesize that the inconsistency is partly due to confounding by circulating melatonin.

Melatonin is a hormone excreted by pineal gland of animals and humans with a marked circadian rhythm.³⁷ It is a broad-spectrum antioxidant and a free radical scavenger and, hence, may affect biomarkers of oxidative stress.⁸⁸ It has been reported in a previous study that melatonin could attenuate oxidative stress and injury induced by cigarette smoking.¹¹⁸ Melatonin also plays a key role in repairing damaged DNA segment and hence may affect urinary excretion of 8-OHdG.¹¹⁹ A recent study reported that compared with day-shift workers, night-shift workers had lower urinary 8-OHdG concentrations and lower circulating melatonin levels.¹²⁰ A lower urinary 8-OHdG level,

in this case, might not indicate a lower level of oxidative stress, because the diminished melatonin level might have decreased the repair of oxidized DNA leading to less 8-OHdG coming off the damaged DNA strand.⁹⁰ Therefore, reporting MDA and 8-OHdG without considering the influence of melatonin, as commonly done previously, may not accurately reflect the 'original' ROS damages to lipids and DNA, because MDA and 8-OHdG excreted in the urine are the net result of the original ROS damage and antioxidant scavenging as well as DNA repair in the case of 8-OHdG. As a major metabolite of melatonin, urinary 6-sulfatoxymelatonin (aMT6s) concentration has been used as a surrogate of circulating melatonin level.⁴⁰ To examine our hypothesis about potential confounding of aMT6s, we utilized the banked urine samples collected in two previous studies that had originally investigated the effects of air pollutant exposure on cardiovascular and lung function.^{14,71}

3.2 Methods

Urine samples and relevant exposure data were obtained from two studies, namely the Shanghai Study and the Changsha Study, which are described in detail previously.^{14,71} Below we describe the information pertinent to the present study.

3.2.1 Study subjects and urine samples

The Shanghai Study enrolled 70 young adults from a pool of medical and nursing trainees living and studying on a hospital campus in a suburb of Shanghai, China. All the subjects were free from chronic diseases and were never smokers. During

the period from November 7 to December 13, 2015, 8 urine samples were collected from each subject including 6 early morning voids (7:00-8:00) and 2 evening voids (18:00-21:00) on 6 different days. The Changsha study enrolled 89 office workers of a company located in a suburb of Changsha City, Hunan Province, China. All the subjects were free from chronic diseases and include self-reported smokers and nonsmokers. From December 2, 2014, to January 30, 2015, up to 4 early morning (7:00-9:00) urines was provided by each subject on 4 separate days. No urines were collected during other time of the day in the Changsha study. All urine samples were aliquoted in sterilized plastic tubes and stored at -80°C before analysis. The protocols of both studies were approved by the Ethics Committee of Shanghai First People's Hospital and agreed by the Duke University Campus Institutional Review Board (Protocol number: 2017-1051 and 2017-0993).

3.2.2 Measurements of MDA, 8-OHdG, and aMT6s

Urinary 8-OHdG and aMT6s were simultaneously analyzed using a high pressure liquid chromatography-tandem mass spectrometry (HPLC-MS-MS) method published recently.¹²¹ In brief, the method involved a liquid-liquid extraction using 20% methanol at urine pH~7; and the extracts were analyzed using the negative ion mode of the mass spectrometer. Urinary MDA concentrations were measured using a method previously reported.¹¹² Briefly, urine samples were derivatized with phosphoric acid and thiobarbituric acid (TBA) at 80 °C. Resulting MDA-TBA adduct was then analyzed using

an HPLC-fluorescence technique. Concentrations of all these three compounds were normalized by creatinine to correct urine dilution. Urinary creatinine was measured using a Creatinine (urinary) Colorimetric Assay Kit (Cayman Chemical, item No. 500701) and a microplate reader.

3.2.3 Assessment of air pollutant exposure

In the Shanghai study, all subjects lived in dormitories located on the hospital campus where they also worked and studied. PM_{2.5} was monitored inside and outside subjects' dormitories, inside a representative classroom and a clinical office. Ambient NO₂ concentration (Thermo 42i NO-NO₂-NO_x chemiluminescence gas analyzer, Thermo Scientific, USA) along with temperature and relative humidity (WSZY-1, Beijing Tian Jianhuayi Technology Development Co., Ltd) were monitored on the third floor of a building approximately 200m from the study site. Ambient ozone (O₃) was obtained from the nearest governmental environmental monitoring station located at 16 km northwest of the hospital campus. The O₃ measured by the monitoring station could be a good estimation of the ambient O₃ level at the hospital campus for the following reasons. Firstly, the prevailing wind direction was from northwest of Shanghai during the study period. Secondly, there were no industrial sources in between the monitoring station and study site, both located in the suburb of Shanghai. Because O₃ and NO₂ were not measured inside dorms, offices, and classrooms, we estimated indoor concentrations using outdoor concentrations and indoor/outdoor (I/O) ratios established through our

measurements of ventilation rate for the dormitory rooms or those published for similar building conditions. We used I/O ratios of 0.8 for PM_{2.5}, 0.35 for O₃, and 0.8 for NO₂⁷¹. In the Changsha study¹⁴, the subjects lived in company-provided dormitories and worked in office buildings during weekdays. Indoor PM_{2.5} and O₃ were measured inside subjects' dorms and offices. Ambient PM_{2.5}, O₃, and NO₂ concentrations, along with temperature and humidity, were obtained from the nearest governmental monitoring station (~4.5 km from the company grounds). Indoor concentrations for the microenvironments without actual measurements were estimated using established I/O ratios similar to the Shanghai study. Based on detailed time-activity data collected on each subject in both studies and pollutant concentrations measured or estimated for each encountered microenvironment, we calculated personal air pollutant exposures averaged over the 12-hour and 24-hour periods prior to urine collection. These are termed 12h and 24h pollutant exposure, respectively.

3.2.4 Statistical analysis

Based on data distributions, concentrations of the 3 urinary compounds were natural logarithm-transformed. First, we used linear mixed-effects regression (LMER) models to assess the association of aMT6s with 8-OHdG and MDA, respectively. In these models, 8-OHdG or MDA concentration was the dependent variable, and aMT6s was independent variable. In these models, we controlled for 24-hour ambient temperature and relative humidity as covariates to adjust for the outdoor weather condition, which

might affect aMT6s level and biomarkers of oxidative stress. In addition, we also controlled for age, sex, smoking, menstruation, and respiratory infection, which have been previously associated with circulating melatonin level and systematic oxidative stress. The 'study' factor (Shanghai versus Changsha study) was controlled to adjust for potential influence introduced by different studies. Subject ID and the day of week for urine collection were controlled as random-effect variables. From the model output, we calculated percent change (and 95% confidence interval) in 8-OHdG or MDA concentration associated with an interquartile range (IQR) increase in aMT6s.

Second, we used the LMER models to examine the association of 12h and 24h pollutant exposure, respectively, with aMT6s. In this set of models, aMT6s was dependent variable, and pollutant exposure was an independent variable. In addition to the fixed-effect covariates described above, time of day for urine collection (morning versus evening) was added as another covariate. Random-effect variables were the same. We reported percent change (95% CI) in aMT6s concentration associated with an IQR increase in pollutant exposure.

Third, we used the LMER models to assess the pollutant exposure-response relationships for 8-OHdG and MDA with and without controlling for aMT6s. In these models, 8-OHdG or MDA concentration was the dependent variable, and each of air pollutant exposure was the independent variable along with the same covariate structure in the first model described above. Controlling for aMT6s was conducted by

adding aMT6s as a covariate in the models. From each model output, we generated estimates in percent change (95% CI) in 8-OHdG or in MDA associated with an IQR increase in pollutant exposure.

Forth, we conducted stratified analyses to assess pollutant exposure-response relationships for 8-OHdG and MDA based on high (> median) versus low (\leq median) aMT6s levels. Similarly, 8-OHdG or MDA concentration was the dependent variable, and each of air pollutant exposure was the independent variable. The same covariate structure as described in the first model was adjusted with aMT6s controlled as a covariate. From each model output, we generated estimates in percent change (95% CI) in 8-OHdG or in MDA associated with an IQR increase in pollutant exposure.

Finally, we conducted several sensitivity analyses. (1) We used co-pollutant models to examine whether the exposure-response relationship obtained in the single-pollutant models can be retained after controlling for a co-pollutant. The model structure was the same as that for the single-pollutant models. (2) We conducted analyses by excluding current smokers, subjects undergoing menstruation and respiratory infection during the study period. (3) We conducted separate analyses for the Shanghai and the Changsha studies, respectively, on all the relationships analyzed using the combined dataset.

All statistical analyses were conducted using R software (version 3.3.2), *lme4* and *lmeTest*. P value of 0.05 was set as the cut point for statistical significance. A detailed

description of equations and codes for the statistical models is provided in Supporting Materials.

3.3 Results

Characteristics of the study subjects are summarized in Table 1. Of the total of 159 subjects, 70 (41 male and 29 female) were from the Shanghai study and 89 (52 male and 37 female) were from the Changsha study. The Changsha study included 15 current smokers while the Shanghai study had none. The average age (22.1 years) in the Shanghai study was lower than those (31.5 years) in the Changsha study. Based on the questionnaire survey, none of the subjects reported taking melatonin supplementation during the study period.

Of the 889 total urine samples, 560 were from the Shanghai study and 329 were from the Changsha study. Concentrations of the three urinary compounds are also shown in Table 5 by study and also for the pooled data. Notably the Shanghai subjects had a wider range of aMT6s than the Changsha subjects. This reflects that all the urine samples from the Changsha study were collected in the early morning, while the Shanghai study collected 420 early morning voids and 140 evening voids. As expected, melatonin had a diurnal pattern, with markedly higher concentrations of aMT6s in the early morning urines (15.1 ± 9.0 ng/mg creatinine) than in the evening urines (5.6 ± 5.3 ng/mg creatinine) (t-test, $p < 0.001$). In addition, although aMT6s was measured in both early morning and evening urine in the Shanghai study, the average aMT6s level in the

Shanghai study (12.7 ± 9.3 ng/mg creatinine) was still slightly higher than that in the Changsha study (11.6 ± 9.2 ng/mg creatinine) because the younger participants in the Shanghai study had higher 'baseline' circulating melatonin levels ¹²².

Table 5. Subject characteristics and urinary concentrations of aMT6s, 8-OHdG, and MDA.

Subject Characteristics		
	Value	
Participant, No. (%)		
Shanghai	70 (44.0%)	
Changsha	89 (56.0%)	
Pooled	159	
Female, No. (%)		
Shanghai	29 (41.4%)	
Changsha	37 (41.6%)	
Pooled	66 (41.5%)	
Current smoker, No. (%)		
Shanghai	0	
Changsha	15 (16.9%)	
Pooled	15 (9.4%)	
Age, mean \pm SD [range], year		
Shanghai	22.1 ± 1.6 [19-26]	
Changsha	31.5 ± 7.6 [22-52]	
Pooled	27.4 ± 7.5 [19-52]	
Urinary Biomarkers		
	Mean \pm SD	Median [range]
aMT6s (ng/mg creatinine)		
Shanghai	12.7 ± 9.3	10.8 [0.4-93.5]
Changsha	11.6 ± 9.2	8.8 [0.3-55.3]
Pooled	12.3 ± 9.2	10.3 [0.3-93.5]
8-OHdG (ng/mg creatinine)		
Shanghai	2.9 ± 4.6	1.9 [0.3-67.2]
Changsha	4.0 ± 4.1	3.0 [0.4-42.8]
Pooled	3.3 ± 4.5	2.3 [0.3-67.2]
MDA (ng/mg creatinine)		
Shanghai	78.1 ± 55.3	63.4 [1.1-558.1]
Changsha	68.1 ± 51.1	55.9 [4.7-613.6]
Pooled	74.4 ± 53.6	60.9 [1.1-613.6]

Estimates of personal air pollutant exposure averaged over the 12-hour and 24-hour period prior to urine collection are shown in Table 6. On average, 12h PM_{2.5}, O₃, and NO₂ in the Shanghai study were lower than those in the Changsha study. Similarly, average 24h PM_{2.5}, O₃, and NO₂ personal exposures were also lower in the Shanghai study. Across all the subjects in both studies, PM_{2.5} exposures were negatively correlated with O₃ exposure (Pearson r: -0.19 for 12h exposures and -0.3 for 24h exposure) and positively correlated with NO₂ exposure (Pearson r: 0.33 for 12h exposures and 0.32 for 24h exposures). The Pearson correlation coefficients (r) between NO₂ and O₃ were 0.06 for 12h exposures and 0.14 for 24h exposures.

Table 6. Personal pollutant exposures to PM_{2.5}, O₃, and NO₂ averaged over the 12-hour and 24-hour period prior to urine collection.

Pollutant	Mean ± SD	Median [range]
12h		
PM _{2.5} (µg/m ³)		
Shanghai	37.5 ± 15.3	38.7 [1.5-74.7]
Changsha	42.8 ± 35.7	27.4 [2.2-169.1]
Pooled	39.4 ± 25.0	37.4 [1.5-169.1]
O ₃ (ppb)		
Shanghai	3.4 ± 2.8	2.4 [0.1-20.6]
Changsha	5.8 ± 3.8	4.1 [1.3-21.6]
Pooled	4.3 ± 3.4	3.3 [0.1-21.6]
NO ₂ (ppb)		
Shanghai	14.8 ± 7.0	13.5 [3.8-36.5]
Changsha	24.0 ± 8.2	22.4 [12.1-42.0]
Pooled	18.2 ± 8.7	15.4 [3.8-42.0]
24h		
PM _{2.5} (µg/m ³)		
Shanghai	37.1 ± 13.0	36.0 [13.0-81.3]
Changsha	39.2 ± 30.5	27.2 [2.0-152.9]
Pooled	37.9 ± 21.3	33.4 [2.0-152.9]
O ₃ (ppb)		
Shanghai	5.0 ± 3.0	4.1 [0.9-15.4]
Changsha	6.7 ± 4.3	4.5 [1.7-19.3]
Pooled	5.6 ± 3.6	4.4 [0.9-19.3]
NO ₂ (ppb)		
Shanghai	15.1 ± 5.3	14.4 [7.1-29.6]
Changsha	23.6 ± 6.8	23.5 [12.4-39.4]
Pooled	18.3 ± 7.2	16.6 [7.1-39.4]

3.3.1 Relationships between oxidative stress biomarkers and aMT6s

With a wide range of aMT6s (see Table 5), we found that increasing levels of aMT6s were significantly associated with increasing concentrations of both 8-OHdG and MDA. As shown in Figure 4, an interquartile range (IQR, equivalent to 171%) increase in

aMT6s was associated with a 16.1% (95% CI: 9.1%-23.6%) increase in 8-OHdG and an 8.1% (2.7%-13.9%) increase in MDA.

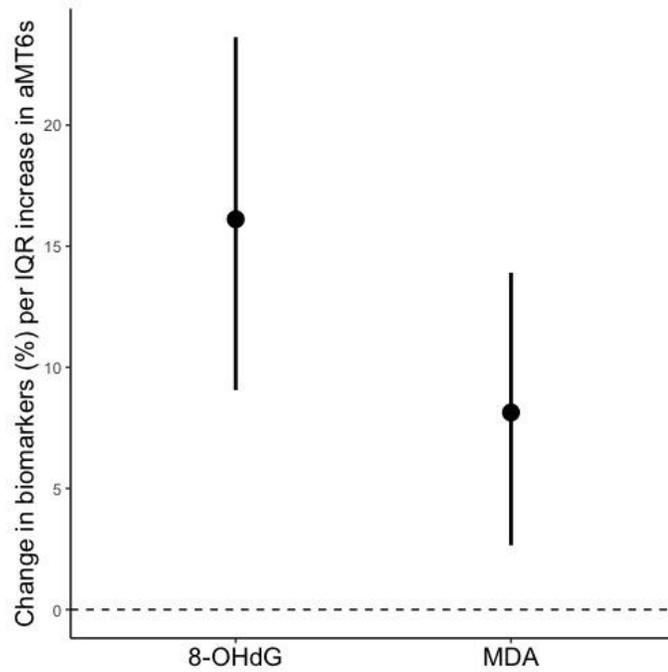


Figure 4. Estimated means and 95% confident intervals for change in biomarkers (%) per IQR increase in aMT6s.

3.3.2 Relationships between aMT6s and pollutant exposures

A previous study suggests that air pollution exposure may affect circulating melatonin levels ¹²³. However, as shown in Figure 5, we did not observe a significant association between aMT6s and any of the 6 pollutant exposure variables (3 pollutants x 2 time period).

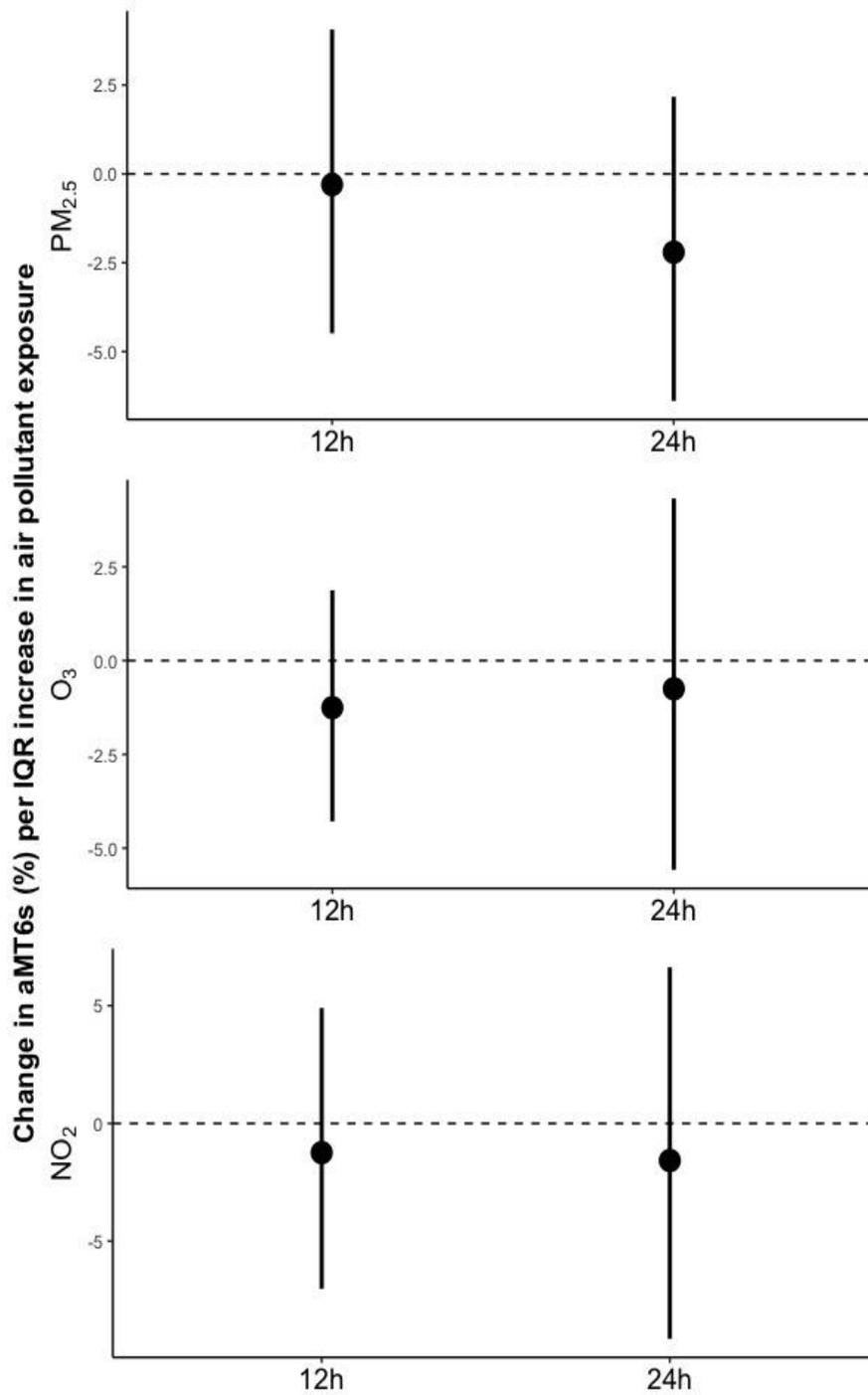


Figure 5. Estimated means and 95% confident intervals for change in urinary aMT6s (%) per IQR increase in PM_{2.5}, O₃, and NO₂ from the single-pollutant LMER models.

3.3.3 Relationships between pollutant exposures and oxidative stress biomarkers with and without aMT6s adjustment

Using individual-level exposure data and biomarker concentrations, we examined the exposure-response relationships with and without adjusting for aMT6s. Results are shown in Figure 6. Without adding aMT6s in the LMER model, we did not observe significant pollutant-biomarker associations except a negative association between O₃ (both 12h and 24h exposure) and MDA. Adding aMT6s as a covariate in the LMER models resulted in significant and positive associations between 12h PM_{2.5} and MDA, between 12h O₃ and 8-OHdG, and between 12h NO₂ and MDA. An IQR increase in PM_{2.5} exposure (29.0 µg/m³) was associated with a 6.1% (95%CI: 1.6%-10.8%) increase in MDA. An IQR increase in O₃ exposure (2.7 ppb) was associated with a 5.7% (1.9%-9.7%) increase in 8-OHdG. An IQR increase in NO₂ exposure (10.4 ppb) was associated with an 8.6% (1.3%-16.5%) increase in MDA. In addition, the significant negative association between 12h O₃ exposure and MDA observed in the unadjusted model now became non-significant. In contrast, aMT6s adjustment made little difference in pollutant exposure associations with MDA or 8-OHdG.

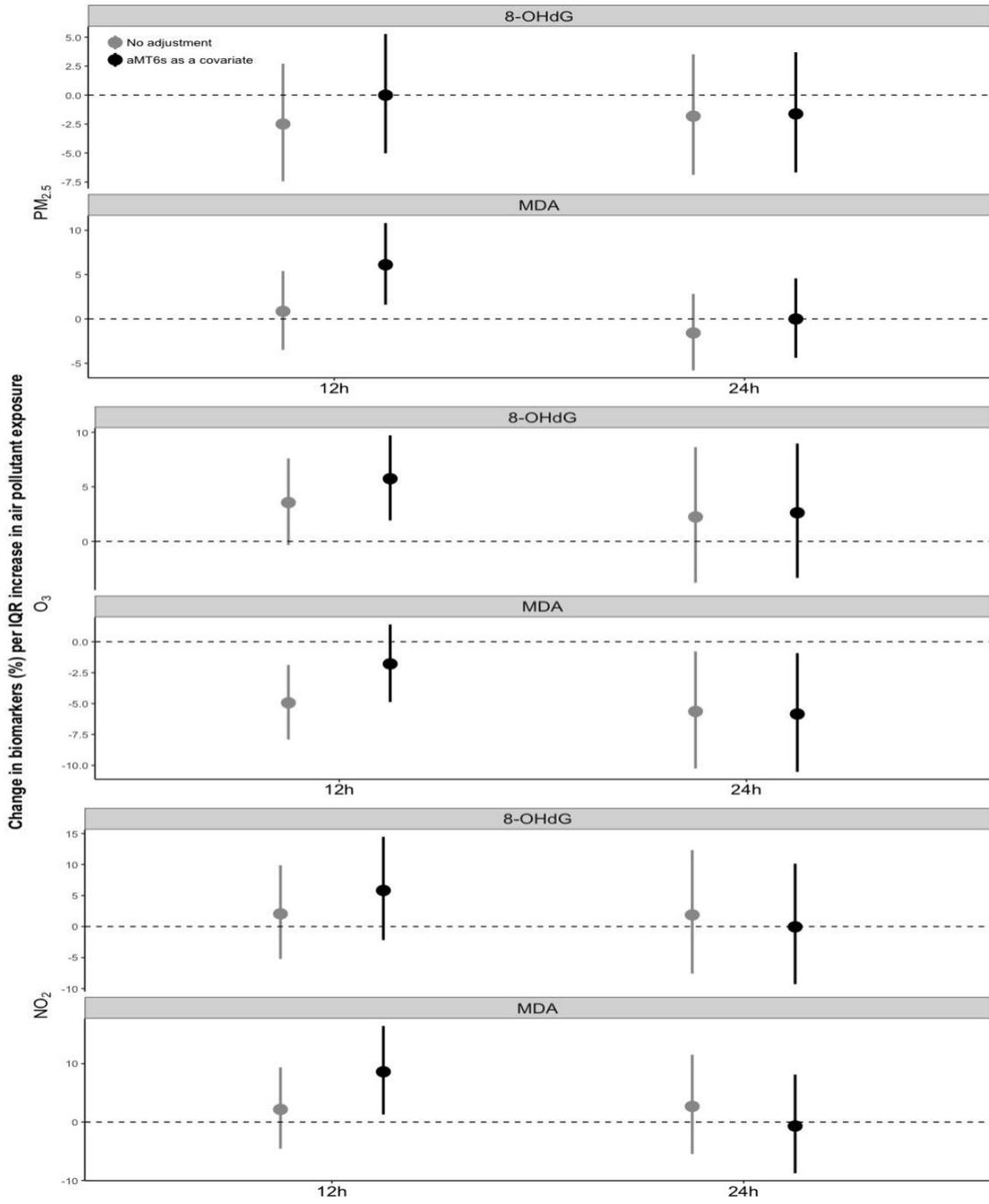


Figure 6. Estimated means and 95% confident intervals for change in biomarkers (%) with one IQR increase in PM_{2.5}, O₃, and NO₂.

3.3.4 Exposure-response relationships stratified by aMT6s level

To examine whether aMT6s modifies the relationship between air pollutant exposure and the 2 biomarkers of oxidative stress, we conducted a stratified analysis with the cut point of median aMT6s level. As shown in Figure 7, we found that the significant positive associations of MDA with 12h O₃ and NO₂ in the low aMT6s group were changed to be non-significant in the high aMT6s group, accompanied by a remarkable decrease in effect size. The effect of 12hr O₃ on 8-OHdG was more evident in the high aMT6s group.

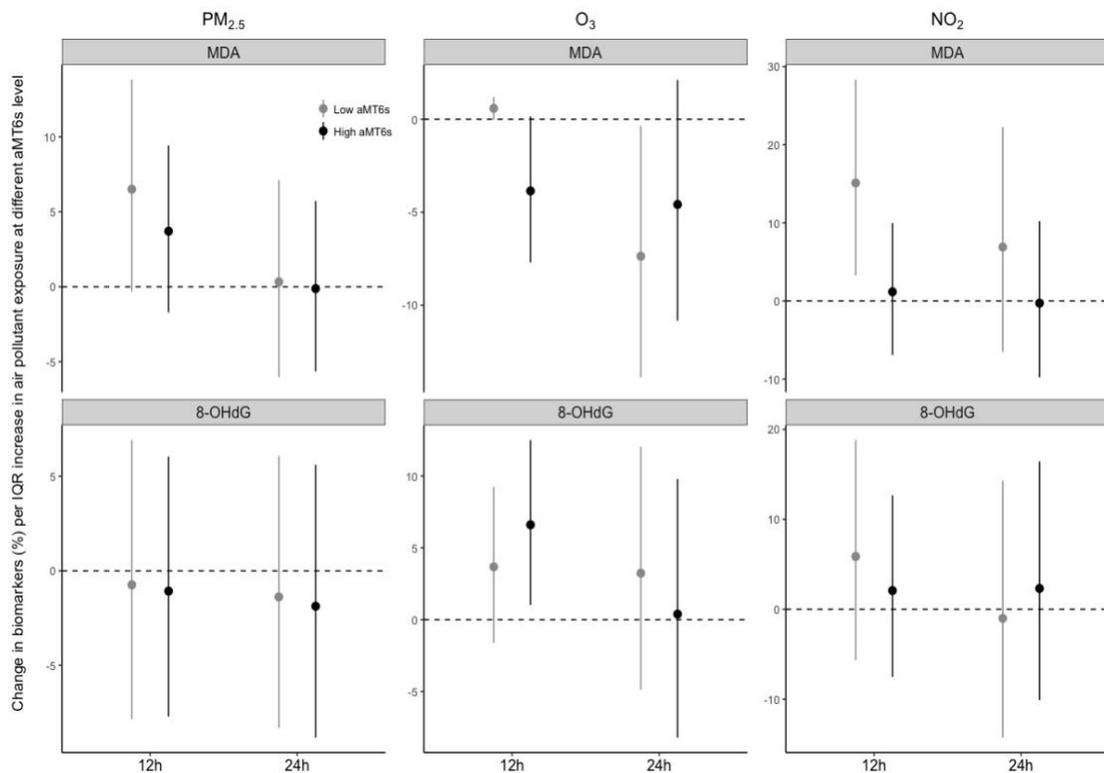


Figure 7. Change in biomarkers (%) with one IQR increase in PM_{2.5}, O₃, and NO₂. Low aMT6s group: ≤10.3 ng/mg; high aMT6s group: >10.3 ng/mg.

3.4 Discussion

The positive association of aMT6s with 8-OHdG was consistent with that observed in previous studies. One study reported that compared with healthy controls, women with polycystic ovarian syndrome (PCOS) had both higher urinary 8-OHdG concentrations and higher aMT6s levels.¹²⁴ The authors attributed higher 8-OHdG concentrations in the PCOS women to increased body burden of oxidative stress. They attributed higher aMT6s levels in the same women to increased melatonin production stimulated by higher oxidative stress. This stimulation process could be supported by a recent finding that melatonin could be synthesized in mitochondria in response to the increase in oxidative stress.¹²⁵ Another study found that compared with day-shift workers, night-shift workers had both lower 8-OHdG and lower aMT6s levels.^{120, 126} Although the explanation offered to the PCOS women study can also be used to explain the finding in the shift workers study, the authors of the latter study offered alternative explanations as to why the night-shift workers, who were expected to have had a higher level of mental stress and oxidative stress, had lower 8-OHdG concentrations. Bhatti et al (2017) explained this from the standpoint of melatonin's role in repairing damaged DNA.¹²⁰ Night-shift workers had diminished melatonin production, leading to diminished amount of ROS-oxidized DNA strands being repaired. During the repairing process, 8-OHdG was released from the damaged DNA strand and then excreted in the urine. Hence although the original ROS concentrations were perhaps higher in the night-

shift workers, the excretion of 8-OHdG molecules may have been decreased due to diminished amount of melatonin to help repair ROS-DNA adducts. We think both of these previously hypothesized mechanisms can be used to explain the positive association between urinary aMT6s and 8-OHdG observed in our study.

It appears that no previous studies have examined the relationship between urinary MDA and aMT6s. The positive association between aMT6s and MDA observed in the present study is somewhat supported by a previous study that showed a significant positive association between serum melatonin and serum MDA in patients with traumatic brain injury.¹²⁷ Our explanation is similar to one of the explanations for the 8-OHdG and aMT6s relationship. Increasing oxidative stress could have stimulated melatonin generation via body's defense mechanism to scavenge the excess production of ROS.

None of our study subjects self-reported taking melatonin supplementations. This was confirmed by the measurement data, as urinary aMT6s concentrations of these subjects were far below the levels reported following taking a dose of supplementation. For example, one study observed a 160-fold increase in aMT6s within one hour following a single 3 mg dose of melatonin.⁴¹ In human subjects taking melatonin supplementations, the ROS stimulation-based mechanism, as observed in the present study, has been overwhelmed by melatonin's direct ability to scavenge ROS. In these studies, following a dose (range 3 – 10 mg per day) of melatonin supplementation,

urinary MDA concentration significantly decreased by 27%-56%.¹²⁸⁻¹³⁰ This is due to rapid and substantial increase in the amount of circulatory melatonin that rapidly depleted ROS, leading to decreased lipid peroxidation. However, for the ROS-scavenge mechanism to be dominant, it appears aMT6s levels need to be substantially higher than the natural range of (0-110.8 ng/mg creatinine) without melatonin supplementation.^{107, 131}

It has been widely accepted that air pollution exposure increases oxidative stress, as inhaled pollutants can induce or enhance the production of reactive oxygen species (ROS) in the respiratory tract and spill over to the circulatory system. Urinary 8-OHdG and MDA, resulting from ROS reactions with DNA and lipids, respectively, are established biomarkers of oxidative stress. However, the relationships between air pollutant exposure and urinary 8-OHdG and MDA have not been consistently observed among published studies. In the present study, our main motivation was to examine melatonin as a potential factor that may have contributed to the inconsistency. We found that aMT6s (a urinary metabolite and surrogate of circulatory melatonin), with known substantial diurnal variations, may have confounded the associations between air pollution exposure and the two urinary biomarkers of oxidative stress. As shown in Figure 6, without controlling for aMT6s, there was not a clear pattern in the relationships, which is consistent with some of previous studies.¹¹⁵⁻¹¹⁷ By controlling for aMT6s in the LMER models, we found significant positive associations between 12h PM_{2.5} exposure and MDA, 12h O₃ exposure and 8-OHdG, and 12h NO₂ exposure and

MDA. Furthermore, we found that aMT6s adjustment affected the relationship with 12h pollutant exposure but not 24h exposure. This may reflect the fact that circulatory melatonin has a short-half life (59-65 minutes).¹³² Hence, melatonin's actions to affect 8-OHdG and MDA excretion would occur within a shorter period of time.

In our attempt to examine whether aMT6s mediated the association between air pollutant exposure and oxidative stress biomarkers, we assessed the relationship between pollutant exposure and aMT6s, finding no significant associations of aMT6s with either 12h and 24h air pollutant exposure (see Figure 5). There have been no previous studies reporting the effects of 12h or 24h air pollution exposure on urinary aMT6s. However, a previous study showed that increased 9-day average PM_{2.5} exposure was significantly associated with increasing serum melatonin levels in healthy adults in Shanghai.¹²³ Although it is hard to compare our study with this previous study of different study design and exposure duration, we offer two reasons for the difference in study findings. (1) Compared to longer-term PM_{2.5} exposure, the ROS induced by acute (12h and 24h) exposure may not last long enough to affect circulating melatonin level. (2) In the previous study, melatonin was measured in the blood collected during the daytime, and hence may not reflect the main production of this molecule during the nighttime. It is known that melatonin is metabolized in the liver rapidly and exhibits a short half-life of approximately 1 hour. In the present study, we measured aMT6s, the primary urinary metabolite of melatonin, reflecting the amount of nocturnal plasma

melatonin in the body.⁴⁰ Nevertheless, the lack of a significant association between air pollutant exposure on aMT6s suggests that aMT6s at naturally occurring level (i.e., no supplementation) might not mediate the association between short-term pollutant exposure and the two oxidative stress biomarkers. However, the current study lacks the ability to confirm such a mediation effect, given that melatonin secretion was high during the night, while pollutant exposure during sleeping hours (indoors) was low. This makes it harder to evaluate the likelihood for air pollutant exposure to cause aMT6s changes that consequently affect MDA and/or 8-OHdG.

In our attempt to examine whether aMT6s modified the relationships between pollutant exposure and the oxidative stress biomarkers, we assessed the interaction between aMT6s and pollutant exposure in the LMER models and also conducted aMT6s-stratified analyses. The aMT6s by exposure interaction was not significant for all three pollutants, except for 24h O₃ exposure (Table B1). However, the main effect of 24h O₃ in the models was not significant. Results from the stratified analyses (Figure 7) suggest that aMT6s may modify the short-term (12hr) MDA effects by O₃ and NO₂ and the short-term 8-OHdG effects by O₃, respectively. Taking the interaction analysis and stratified analysis together, we did not observe strong evidence to support a modification effect of aMT6s.

It is well known that light/dark cycle affects melatonin levels dramatically. Lacking the direct measurement of light exposure may be a limitation of this study.

However, the influence of light exposure on the study findings may be minimized by the following features of the study design and data analysis. (1) All the subjects were living and working in either hospital or company campus with a relative uniformity of lifestyle. (2) Our statistical models controlled for ambient temperature and relative humidity as indicators of outdoor weather, which could also serve as a proxy for outdoor light exposure. (3) In considering the potential effects of weekday and weekend on subjects' sleep duration, we controlled the day of week for urine collection as a random-effect variable. (4) We used linear mixed-effects models with subject ID as the random effect to address the random intercepts of different subjects with potential differential light exposures.

3.5 Sensitivity analyses

We evaluated the robustness of the results through three sensitivity analyses. Firstly, we investigated whether the relationships observed in the single-pollutant models can be retained after controlling for a co-pollutant. After controlling for any of two co-pollutants, the relationship between 12h air pollutant exposure and aMT6s was not markedly changed in terms of either statistical significance or effect size (Figure B1). Similarly, there were no noticeable changes in the association of 12h pollutant exposure with 8-OHdG or MDA adjusting for aMT6s after controlling for a co-pollutant, except that the non-significant positive association between 8-OHdG and NO₂ became significant after controlling for O₃ and the significant positive association between MDA

and 12h NO₂ became non-significant after controlling for PM_{2.5} (Figure B2). Secondly, after excluding current smokers, subjects undergoing menstruation, and those reporting respiratory infection, the analyses showed similar results (see Figure B3-B6), supporting the robustness of the main analysis findings. Finally, all the relationships analyzed using the combined dataset were evaluated using separate analyses for the Shanghai subjects and the Changsha subjects, respectively. No marked changes were found for the association of aMT6s with 8-OHdG or MDA (Figure B7) and the association between 12h air pollutant exposure and aMT6s in each of studies (Figure B8). Similarly, the associations of 12h air pollutant exposure with 8-OHdG or MDA without adjusting for aMT6s were not noticeably changed. However, some of the significant biomarker-pollutant relationships, with controlling for aMT6s, shown in the combined dataset were retained in the Shanghai subjects but not in the Changsha subjects (Figure B9). The discrepancy may be due to reduced sample size and statistical power in the separate analyses. In addition, only morning urines were collected in the Changsha subjects, showing a narrower range of aMT6s concentration than in the Shanghai subjects and in all the subjects combined.

3.6 Conclusions

Urinary aMT6s concentrations exhibited a wide range in healthy adults, as a result of natural endogenous melatonin metabolism, although substantially lower compared to the reported aMT6s levels after having taken melatonin supplementation.

Within this natural range of aMT6s concentrations, increasing aMT6s concentrations were associated with increased urinary 8-OHdG and MDA concentrations. The natural variation in urinary aMT6s confounded the associations of short-term (12 hour) exposure to air pollutants with 8-OHdG and MDA. Future studies in human subjects with a wider range of aMT6s concentrations (including individuals taking or not taking melatonin supplementations) can help further understand the dynamic roles that melatonin plays in regulating oxidative stress processes in the presence of an external stressor such as air pollution exposure.

Chapter 4. The role of melatonin in inflammatory responses to air pollution exposure in healthy adults.

This chapter addresses Aim 3. The Aim 3 study used urine and blood samples, urinary MDA concentration, and relevant exposure data that had been obtained from a previous study.¹⁴ The current work involves the analysis of stored plasma samples for 9 inflammatory cytokines and urine samples for aMT6s. This chapter includes two parts: Part A and Part B.

In Part A, the associations of air pollution exposure with 9 inflammatory cytokines and urinary MDA were examined. In Part B, urinary aMT6s was associated with both air pollution exposure and the inflammatory cytokines. In addition, the mediation effects of urinary aMT6s on the relationships of air pollution exposure and inflammatory cytokines were investigated.

4.1 Part A: Inflammatory and oxidative stress responses of healthy adults to changes in personal air pollutant exposure.

Part A is adapted with permission from Hu, X.; He, L.; Zhang, J.; Qiu, X.; Zhang, Y.; Mo, J.; Day, D. B.; Xiang, J.; Gong, J., Inflammatory and oxidative stress responses of healthy adults to changes in personal air pollutant exposure. *Environmental Pollution*. 2020, 263 (Publisher: Elsevier). X. Hu and L. He are co-first authors on the publication. Specifically, I (L. He) contributed to research idea conception, statistical analyses, results interpretation, tables and figures design, and drafting and editing of the manuscript. The accompanying supporting information is included in Appendix C. The coauthors contributed to the study design (JG and JZ), manuscript revisions (JG, JZ and HX), sample and data collection in the original study (YZ, JM, DD, and JX), sample analysis (HX and XQ), and discussions (JG, JZ, and XH).

4.1.1 Introduction

Air pollution exposure is one of the leading risk factors for the global burden of disease.¹³³ It has been widely reported that air pollution exposure was associated with adverse pulmonary and cardiovascular health effects.^{133, 134} However, the molecular-level biological mechanisms underlying these epidemiologic associations are still not adequately understood.

Systemic inflammation has been accepted as one of the common pathophysiologic mechanisms of cardiopulmonary diseases.^{15, 135, 136} to air pollutants may trigger inflammatory responses in the cardiorespiratory system^{137, 138} These

inflammatory responses involve various cytokines. For example, air pollutant exposure can stimulate airway epithelial cells and alveolar macrophages to release inflammatory cytokines (e.g., interleukins IL-1 β , IL-6, and IL-8), consequently increasing the expression of other cytokines in regulating inflammatory responses.¹³⁸ Among these cytokines, IL-1 β , IL-2, IL-6, IL-17A, interferon-gamma (IFN- γ), and tumor necrosis factor- α (TNF- α) mainly regulate the proinflammatory response, while IL-4, IL-10, and IL-13 mainly reflect anti-inflammatory response.¹³⁹⁻¹⁴³ Furthermore, exposure to air pollutants may increase systemic oxidative stress in both the respiratory tract and the circulatory system. Malondialdehyde (MDA), a stable product of lipid peroxidation resulting from reactive oxygen species (ROS), has been commonly used as a biomarker of oxidative stress.¹¹²

Previous epidemiologic studies have investigated the changes in immune-inflammatory and systematic oxidative stress in responses to air pollutant exposure in healthy adults, generating inconsistent findings. For example, a study has reported that sub-chronic ozone (O₃) exposure was associated with increased levels of proinflammatory cytokines.¹⁶ Other studies reported that acute O₃ exposure was non-significantly or negatively associated with proinflammatory cytokines.¹⁴⁴⁻¹⁴⁶ Here, we hypothesize that the immune-inflammatory and oxidative stress responses to air pollutant exposure vary with exposure duration. Exploring this variation is important to

understand the biological mechanisms underlying inflammatory and oxidative stress responses to air pollutant exposure.

To test this hypothesis, we leveraged a study that originally investigated the cardiorespiratory effects of an indoor air quality intervention in healthy adults.¹⁴⁷ In the present study, we specifically analyzed banked plasma samples for a suite of cytokines and used urinary MDA data as well as air pollutant exposure data in the original study. We examined the associations of inflammatory cytokines and MDA with personal exposures, with different averaging times, to air pollutants including O₃, particulate matter with aerodynamic diameter < 2.5 μm (PM_{2.5}), nitrogen dioxide (NO₂), and sulfur dioxide (SO₂).

4.1.2 Method

4.1.2.1 Study subjects and study design

The current study used part of the samples collected from an existing interventional study, and the design of the study can be found elsewhere.¹⁴⁷ Briefly, from December 6th, 2014 to January 13th, 2015, healthy workers were recruited from a company campus in Changsha, China. In the living (the dormitory) and working (the offices) buildings where the participants spent most of their time, a filtration system was installed in a central air handling unit (AHU). The AHU was composed of a pre-filter (for coarse particles), an electrostatic precipitator (ESP), and a high-efficiency particulate air (HEPA) filter, which had PM_{2.5} removal efficiencies of approximately 50%, 60%, and >

99%, respectively. However, during the running of an ESP, a byproduct, O₃, was generated, corresponding to a 3.0-10.0 ppb increase in the steady-state concentration of indoor O₃.¹⁴ Among the subjects recruited in the original study, 53 received intervention only on the ESP but not the pre- or HEPA filters. For the rest of 36 subjects, both the ESP and the HEPA filter were manipulated.¹⁴

In the current study, in order to determine the effects of the ESP removal on inflammatory and oxidative stress responses and concerning the constraints of funding and resources, 53 subjects receiving the intervention only on the ESP were analyzed. The current sample size was able to obtain a statistical power of 0.99 based on a priori power test using MANOVA model (see Supplemental Material). Specifically, all the three components of AHU were turned on during pre-intervention period, while the ESP was turned off but the pre- and HEPA filters remained on during the intervention. The timeline of the intervention and the sample collection were shown in Figure 8. In the current study, we measured cytokines from plasma samples and MDA from urine samples collected in three clinic visits implemented in the pre-intervention (Visit 1) and during-intervention (Visits 2 and 3) periods. The first biospecimen collection was conducted 5 days before the ESP removal, and the other two collections were conducted 17 to 38 days after the ESP removal. All the blood and urine samples were collected at the same time in early morning and stored at -80 °C before the analysis.

The study protocol was approved by the Ethics Committee of the Shanghai First People's Hospital and the Campus Institutional Review Board of Duke University. Before inclusion, explicit written informed consent was obtained from all participants, and the participants could withdraw from the study at any time.

4.1.2.2 Air pollutant exposure assessment

Indoor PM_{2.5} and O₃ concentration were continuously monitored in two involved offices during the day and in two dormitory rooms per night using field-calibrated nephelometers for PM_{2.5} concentration measurement (SidePak AM510; TSI Inc) and a UV absorption monitor for O₃ concentration measurement (Model 205; 2B Tech). Ambient PM_{2.5}, O₃, NO₂, SO₂, relative humidity, and temperature were monitored at a government station located 4.5 km from the study site. These measurements were used to establish indoor/outdoor (I/O) ratios, taking into account both filtration conditions and indoor sources, which were later used to estimate hourly averages for PM_{2.5} and O₃ concentrations in known environments (offices and dorms at company campus) when there was no actual measurement. For unmonitored indoor environments (other than the previously mentioned offices and dorms), we used 0.80 and 0.35 for PM_{2.5} and O₃ I/O ratios, respectively, according to previous findings in lightly sealed indoor spaces.¹⁴⁸⁻¹⁵⁰ For NO₂ and SO₂ in all micro-environments, we used 0.80 and 0.50 as the I/O ratio, respectively, based on the physical characteristics of the offices and dorms coupled with previous literature.^{151, 152} These measurements were combined with the detailed time-

activity pattern data collected on each subject to calculate the personal air pollutant exposure averaged over the 12 hours (12 h), 24 hours (24 h), 1 week (1 w), and 2 weeks (2 w) prior to each of biospecimen collection. The questionnaires used to collect the time-activity patterns were shown in the supplementary material. The detail of air pollutant exposure assessment was reported in a previous study.¹⁴

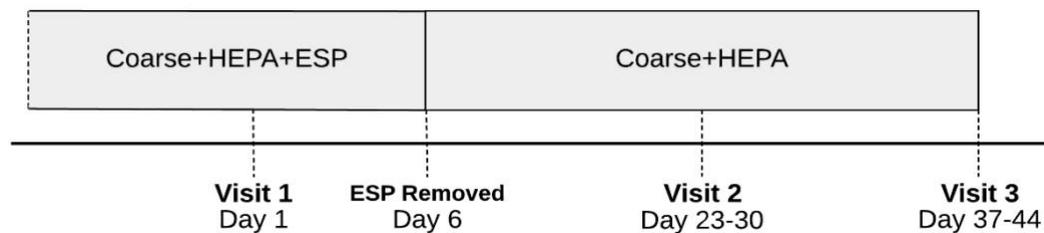


Figure 8. Intervention and visit timeline.

4.1.2.3 Outcome measurement

We analyzed 10 cytokines from the plasma samples using a commercially available analyzing kit (Human Cytokine/Chemokine Magnetic Bead Panel) provided by Millipore Corporation, MA, USA. In brief, plasma aliquots (50 μ L) were firstly centrifuged at 13,000 g for 10 min. Then, 25 μ L of supernatant was collected to measure the concentrations of IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, IL-17A, IFN- γ and TNF- α using Flex MAP 3D™ (Merck Millipore). Milliplex Analyst software (Version 5.1) (Merck Millipore) was used to calculate the concentrations based on a three-parameter standard curve. The limits of detection (LOD) for IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, IL-17A, IFN- γ , and TNF- α were 0.8, 1.0, 4.5, 0.9, 0.4, 1.1, 1.3, 0.7, 0.8 and 0.7 pg/mL, respectively. We assayed 39 pairs of blinded duplicates within the same batch and 7 pairs of blinded

duplicates across different batches to evaluate assay reproducibility, reporting coefficients of variation and intraclass correlation coefficients calculated on natural logarithm-transformed cytokine concentrations.¹⁵³

Urinary MDA concentration was measured and normalized by creatinine using a method previously reported.¹¹² The concentration of urinary 6-sulfatoxymelatonin (aMT6s), a surrogate of circulating melatonin, was measured using a previously reported method¹²¹ to adjust for the potential confounding effect of natural variation in melatonin levels on the relationship between air pollutant exposure (< 24h) and biomarkers of oxidative stress.¹⁵⁴

4.1.2.4 Statistical analysis

Concentrations of the 10 inflammatory cytokines were natural logarithm transformed due to the right-skewed distributions. First, we used linear mixed-effects regression (LMER) models to investigate the exposure-response relationships of personal air pollutants exposure with cytokines and MDA. In these models, the concentration of each of the biomarkers was the dependent variable, and each of the personal air pollutants exposure averaged over the past 12h, 24h, 1w, or 2w was the independent variable. In these models, we controlled for weather-related covariates, including ambient temperature and relative humidity, demographic characteristics, including sex and age, health and behavioral factors, including active and passive smoking status, and respiratory infection status during the 1-week prior to each of the

visit. These covariates have been previously reported to be associated with oxidative stress and inflammatory responses.^{14, 147, 154} The 'batch' factor was controlled as a categorical covariate to adjust for the potential influence introduced by different batches. In addition to these covariates, urinary aMT6s was adjusted for examining the relationship of 12 h air pollutant exposure and MDA concentration. The subject ID was used as a random effect to adjust for the participant-specific intercept. We reported the percent change (95% CI) in biomarkers concentration associated with one standard deviation (SD) increase in pollutant exposure.

Second, concerning the correlations between air pollutants, two-pollutant models were developed to examine the robustness of single-pollutant models after controlling for a second pollutant. In these models, the concentration of each of the biomarkers was the dependent variable, and O₃ exposure averaged over the past 12 h, 24 h, 1 w, or 2 w was controlled as the independent variable, respectively. In addition to the covariates described in the first models, we further controlled for PM_{2.5}, NO₂, and SO₂, respectively, as a second pollutant exposure in these models. Similar two-pollutant models were also developed for PM_{2.5}, NO₂, and SO₂, respectively.

Third, we used LMER models to test the associations of ESP removal with biomarkers. In these models, the concentration of each biomarker was the dependent variable, and the intervention status (before ESP removal vs after ESP removal) was the independent variables of interest along with the covariates (2 w average ambient

temperature and relative humidity, sex, age, smoking status, respiratory infection status, and 'batch' factor). In these models, we further controlled for the variation of 2 w average ambient O₃ concentration when participants were in the unfiltered environment (i.e. anywhere not in the living and working building of the company campus). However, due to the strong negative associations of ambient O₃ with PM_{2.5}, NO₂, and SO₂ concentration during the study period (see Figure C1), we did not control ambient PM_{2.5}, NO₂, and SO₂ concentration when participants were in the unfiltered environment to avoid the multicollinearity issue. Similar to the exposure-response models, urinary aMT6s was adjusted for examining the relationship between ESP removal and MDA concentration. The random effect variable was the same as in the LMER models for the analysis of the exposure-response relationships. We reported the percent change (95% CI) in biomarkers concentrations associated with the ESP removal. Three subjects who were measured only before or after ESP removal were not included in this analysis.

Finally, we conducted the following sensitivity analysis. We examined the exposure-response relationships described in the first models by excluding current smokers and subjects undergoing respiratory infection during the study period. Detailed model results (i.e. effect size, CIs, and p-value) and p-value adjusted by multiple testing using Benjamini-Hochberg methods were shown in Table C1-C2.¹⁵⁵ Statistical analyses were conducted with the R software (version 3.6.1) using the *lme4* and *lmeTest* packages.

P-value of 0.05 was set as the cut-point for statistical significance. The detailed description of statistical methods is provided in the Supplementary Material.

4.1.3 Results

4.1.3.1 Participant characteristics

Of the 53 participants, 16 (30.2%) were women and 11 (20.8%) were current smokers. The participants had a mean (SD) age of 31.4 (7.3) years and a mean BMI (SD) of 22.5 (2.5) kg/m². Among 53 subjects, 52 (98%) completed Visit 1, 47 (89%) completed Visit 2, and 46 (87%) completed Visit 3. Fifty (94%) participants completed at least one visit before the ESP removal and one visit after the ESP removal. Among the total of 145 measurements, 13 (8.97%) reported respiratory infection during the 1-week prior to biospecimen collection.

4.1.3.2 Air pollution exposure

Table 7 summarizes personal air pollutant exposures averaged over 12 hours, 24 hours, 1 week, and 2 weeks prior to each visit for biospecimen collection. Compared to O₃ exposures averaged over the past 12 h, 24 h, 1 w, and 2 w in the pre-intervention period (Visit 1), the O₃ exposures for the corresponding averaging times in the during-intervention period (Visit 2) without ESP running in the AHU were decreased by 69.4%, 62.3%, 54.8%, and 27.0%, respectively. Similar reductions were also found for Visit 3. Compared with the pre-intervention period, the 12 h, 24 h, 1 w, and 2 w personal PM_{2.5} exposures were increased during the intervention period mainly due to the increased

ambient PM_{2.5} concentration. Personal NO₂ and SO₂ exposures for all the four averaging times did not show a notable difference. Ambient air pollution concentrations prior to each visit are shown in Table C3; and the air pollution levels in the indoor environments of the company campus have been in a previous study.¹⁴⁷ The correlations among personal air pollutant exposure are shown in Figure 9. The results indicated that personal O₃ exposure was negatively correlated with personal exposures to PM_{2.5}, NO₂, and SO₂ averaged for each of the four defined periods.

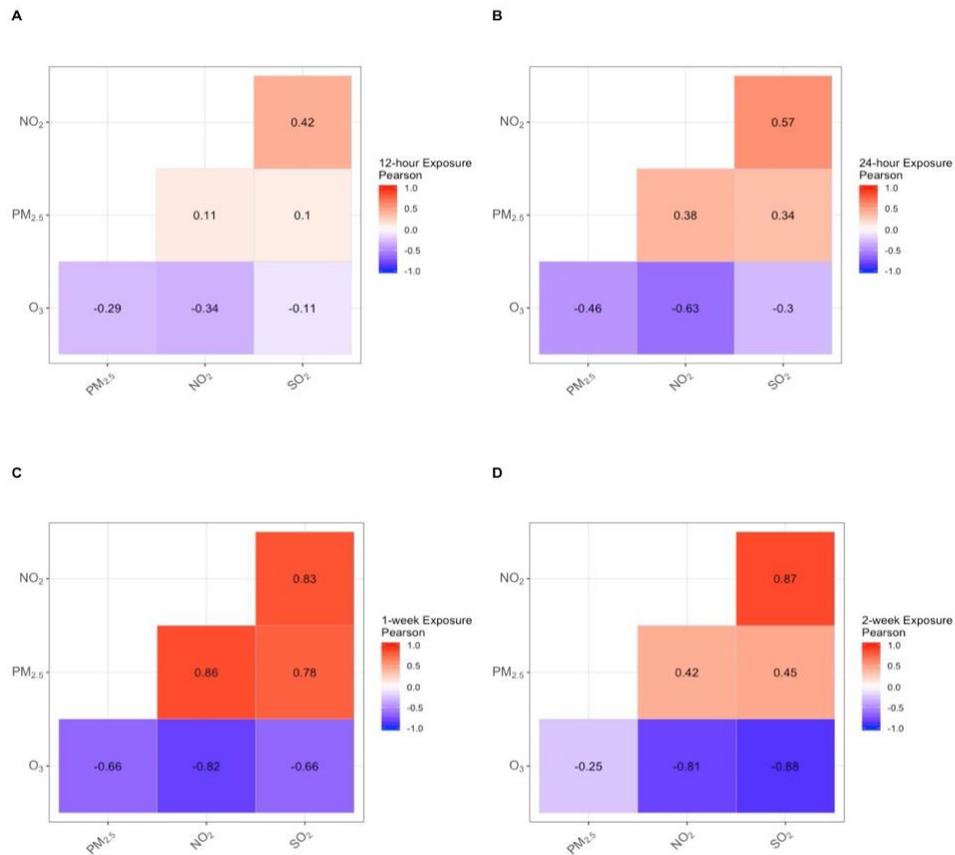


Figure 9. Pearson correlation coefficients among personal air pollutants exposure.

Table 7. The estimated personal exposure to air pollution averaged over 12-hour, 24-hour, 1-week and 2-week before biospecimen collections.

		Pre-intervention (Visit 1)		During-intervention (Visit 2)		During-intervention (Visit 3)		Combined	
		Mean ± SD	Median [Range]	Mean ± SD	Median [Range]	Mean ± SD	Median [Range]	Mean ± SD	Median [Range]
O ₃ (ppb)	12h	12.1 ± 2.5	11.6 [9.9-21.6]	3.7± 1.4	3.4 [1.99-7.07]	4.1 ± 1.66	4.37 [1.44-8.44]	6.9 ± 4.4	5.1 [1.4-21.6]
	24h	13.8 ± 1.9	13.5 [10.6-19.3]	5.2 ± 1.8	4.6 [2.8-10.2]	4.2 ± 1.65	4.26 [1.73-8.82]	8.1 ± 4.7	6.1 [1.7-19.3]
	1w	13.5 ± 0.70	13.5 [11.9-15.9]	6.1 ± 0.69	6.1 [4.7-7.8]	6.6 ± 0.87	6.7 [4.86-9.02]	9.0 ± 3.5	7.2 [4.7-15.9]
	2w	11.2 ± 0.70	11.1 [9.4-13.2]	8.1 ± 1.1	8.0 [5.9-10.8]	6.47 ± 0.56	6.45 [5.47-8.26]	8.7 ± 2.1	8.2 [5.5-13.2]
PM _{2.5} (µg/m ³)	12h	18.4 ± 12.4	15.7 [3.7-48.0]	47.5 ± 36.9	39.2 [4.47-169]	52.9 ± 30.9	48.3 [11.9-124]	38.1 ± 32.0	27.2 [3.7-169]
	24h	15.9 ± 8.0	13.8 [4.5-36.3]	37.3± 22.5	34.5[8.6-112]	47.1 ± 22.7	43.8 [16.1-107]	32.4 ± 22.8	25.6 [4.5-112]
	1w	16.6 ± 3.6	16.1 [11.2-24.6]	30.2 ± 9.4	27.9 [12.1-53.9]	46.6 ± 10.9	43.5 [31.5-79.9]	30.3 ± 15.2	26.9 [11.2-79.9]
	2w	35.2 ± 6.9	35.5 [22.6-49.9]	27.8 ± 6.4	28.2 [13.0-44.1]	47.1 ± 9.63	45.4 [29.4-71.3]	36.5 ± 11.1	35.6 [13.0-71.3]
NO ₂ (ppb)	12h	23.2 ± 2.7	23.8 [16.2-26.6]	33.0 ± 5.8	30.0 [24.4-41.5]	21.8 ± 3.9	20.9 [20.6-41.0]	25.9 ± 6.4	24.0 [16.2-41.5]
	24h	21.4 ± 3.5	20.4 [17.7-27.1]	30.4± 5.8	27.0 [22.8-39.2]	26.6 ± 2.77	25.9 [23.1-30.6]	25.8 ± 5.5	25.8 [17.7-39.2]
	1w	18.0 ± 1.0	18.2 [16.6-19.5]	25.0 ± 2.2	25.0 [21.7-28.1]	30.4 ± 2.95	28.9 [27.8-35.2]	24.1 ± 5.6	25.0 [16.6-35.2]
	2w	23.1 ± 1.3	23.8 [20.9-24.6]	25.3 ± 1.1	25.0 [23.8-27.2]	29.1 ± 1.89	28.4 [27.0-32.2]	25.7 ± 2.9	24.9 [20.6-31.2]
SO ₂ (ppb)	12h	5.6 ± 1.1	5.3 [3.7-9.0]	7.25 ± 1.8	6.87 [4.2-11.8]	5.86 ± 1.69	4.95 [4.07-8.46]	6.2 ± 1.7	5.6 [3.7-11.8]
	24h	5.5 ± 1.0	5.7 [3.6-7.8]	7.8 ± 1.25	7.8 [5.9-10.6]	6.9 ± 2.05	6.29 [4.27-10.2]	6.7 ± 1.8	6.3 [3.6-10.6]
	1w	4.9 ± 0.20	4.9 [4.5-5.5]	6.0 ± 0.52	6.0 [5.0-6.8]	7.38 ± 0.55	7.56 [6.45-8.5]	6.0 ± 1.1	6.0 [4.5-8.5]
	2w	4.5 ± 0.12	4.5 [4.2-4.9]	5.8± 0.26	5.8 [5.3-6.4]	7.13 ± 0.18	7.15 [6.77-7.82]	5.7 ± 1.1	5.8 [4.2-7.8]

Table 8. Summary of cytokines and MDA including pre- and during intervention.

Biomarkers	LOD	Detection Rate	Pre-intervention (Visit 1)		During-intervention (Visit 2)		During-intervention (Visit 3)		Combined	
			Median [IQR]	Range	Median [IQR]	Range	Median [IQR]	Range	Median [IQR]	Range
IL-1 β (pg/mL)	0.8	93%	2.12 [2.57]	0.61-11.2	2.51 [2.35]	0.63-12.6	1.53 [1.58]	0.34-4.82	1.98 [2.33]	0.34-12.6
IL-2 (pg/mL)	1.0	95%	2.1 [1.80]	0.82-22.5	2.7 [2.32]	0.96-8.90	1.74 [1.62]	0.50-7.01	2.25 [1.95]	0.50-22.5
IL-4 (pg/mL)	4.5	50%	3.39 [15.2]	0.01-77.7	5.20 [11.9]	0.10-102	4.86 [27.6]	0.005-146	4.31 [15.9]	0.005-146
IL-6 (pg/mL)	0.9	44%	0.83 [0.86]	0.24-3.56	1.1 [1.33]	0.23-3.62	0.65 [0.585]	0.12-3.8	0.78 [0.81]	0.12-3.8
IL-8 (pg/mL)	0.4	99%	2.29 [1.83]	1.02-21.8	2.02 [2.24]	0.54-31.6	1.49 [2.33]	0.08-14.8	1.9 [2.13]	0.08-31.6
IL-10 (pg/mL)	1.1	64%	1.95 [4.45]	0.12-31.1	3.13 [10.7]	0.08-43.8	1.93 [7.29]	0.02-87.9	2.45 [7.49]	0.02-87.9
IL-17A (pg/mL)	0.7	100%	3.56 [3.94]	1.49-28.5	3.6 [3.25]	1.31-30.8	2.75 [4.55]	0.7-31.2	3.5 [3.95]	0.70-31.2
IFN- γ (pg/mL)	0.8	99%	10.3 [15.7]	2.1-74.2	15.0 [22.2]	1.55-100	11.6 [20.7]	0.79-65.4	12.9 [17.8]	0.79-100
TNF- α (pg/mL)	0.7	99%	13.3 [8.48]	3.73-23.9	14.4 [9.31]	4.94-28.2	8.89 [7.58]	0.50-23.3	11.6 [9.1]	0.5-28.2
MDA (ng/mg creatinine)	NA	100%	51.2 [23.4]	4.7-294	49.3 [37.8]	20.9-439	57.2 [31.5]	30.0-614	51.5 [30.8]	4.7-614

4.1.3.3. Exposure-response relationships: single-pollutant models

The concentrations of the biomarkers are summarized in Table 8. The detection rates for IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, IL-17A, IFN- γ and TNF- α were 93%, 95%, 50%, 44%, 99%, 64%, 18%, 100%, 99% and 99%, respectively. Due to the low detection rate for IL-13, it was excluded from the main analysis. By using pollution exposure and biomarker concentrations, we estimated the exposure-responses associations (Figure 10). The results showed that one standard deviation (SD) increase in the 12h average O₃ exposure (SD = 4.4 ppb) was associated with significant decreases in IL-6, IL-17A, IFN- γ , and IL-10, respectively. One SD increase (4.7 ppb) in the 24 h average O₃ exposure was associated with significant decreases in IL-6, IL-17A, IFN- γ , IL-4, and IL-10, respectively. In contrast, one SD increase in the 1 w average O₃ exposure (3.5 ppb) was associated with significant increases in IL-1 β , IL-2, IL-6, IL-8, and TNF- α , respectively. One SD increase in the 2 w average O₃ exposure (2.1 ppb) was associated with significant increases in IL-1 β , IL-2, IL-6, IL-8, IL-17A, IFN- γ , and TNF- α level, respectively.

The associations of cytokines with personal PM_{2.5}, NO₂, and SO₂ exposure are shown in Figure 10. We found that the associations of the cytokines with personal PM_{2.5}, NO₂, and SO₂ exposures showed an opposite pattern compared to those with personal O₃ exposures. Specifically, we found significant and positive associations of 12 h NO₂ exposure with each of IL-1 β , IL-2, IL-6, IL-8, IL-17A, IFN- γ , and IL-10. Similar

results were also found for 24 h NO₂ exposure. In contrast, we observed significant and negative associations of 1 w and 2 w NO₂ exposures with IL-2, IL-6, IL-8, and TNF- α . Moreover, we found significant and negative associations of pro-inflammatory cytokines with PM_{2.5} and with SO₂, with exposures averaged over 1 week or 2 weeks prior to biospecimen collection.

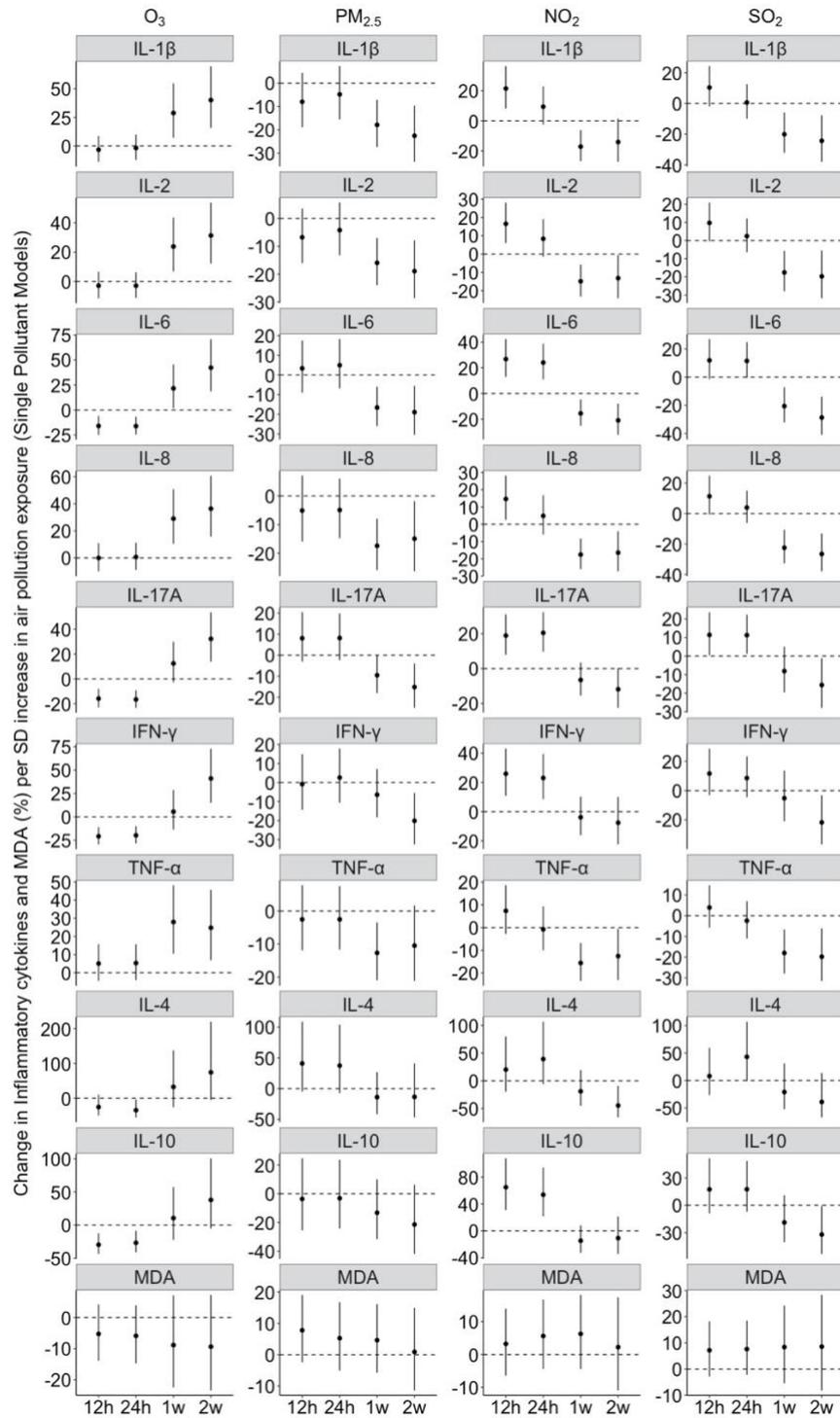


Figure 10. Change in biomarkers (%) with one SD increase in air pollutant exposure, respectively: results from single-pollutant models.

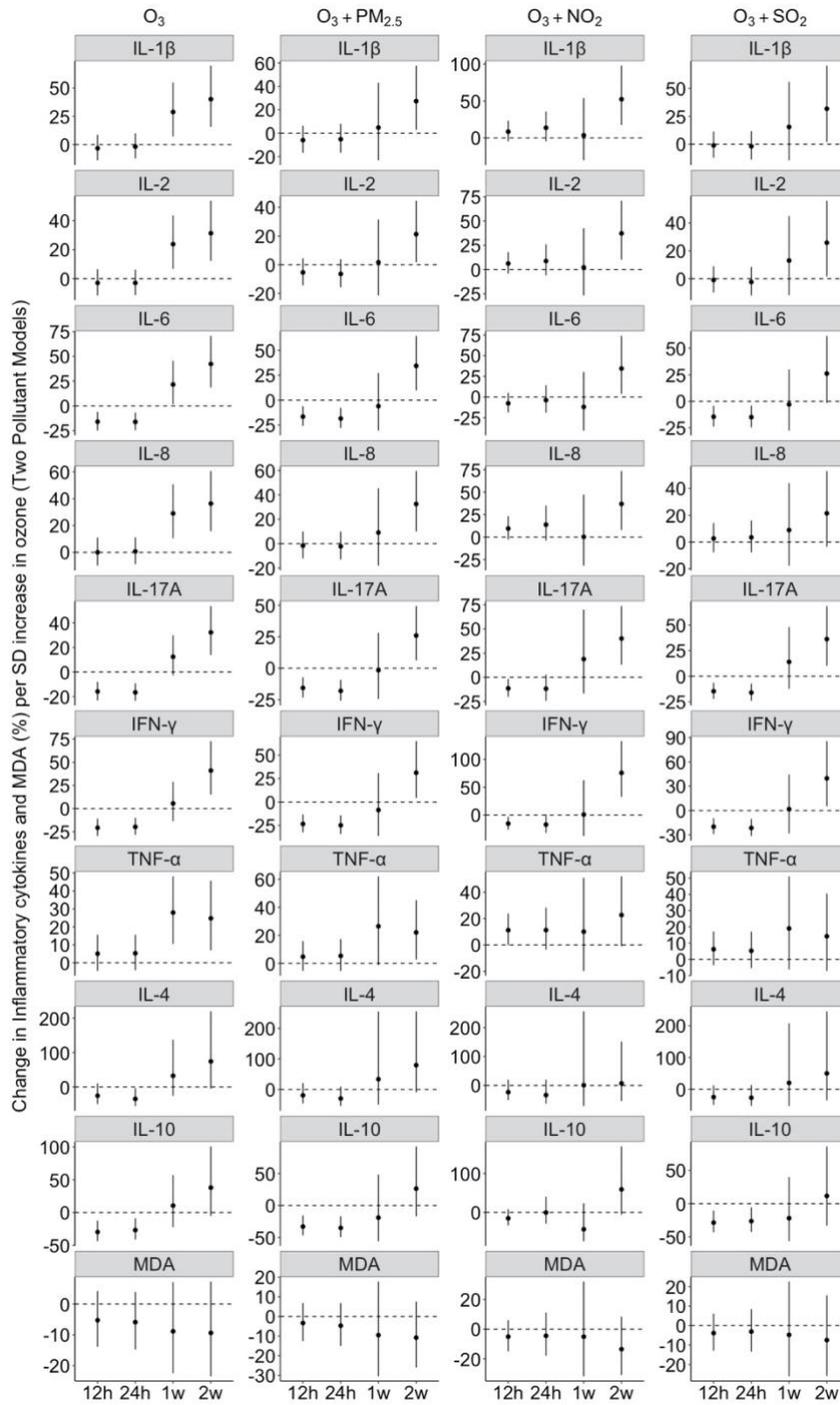


Figure 11. Change in biomarkers (%) associated with one SD increase in O₃ exposure, respectively: results from two-pollutant models.

4.1.3.4 Exposure-response relationships: two-pollutant models

The results of the two-pollutant models are shown in Figure 11. We found that the significant and negative associations of 12 h O₃ exposure with IL-17A and IFN- γ were not noticeably changed after controlling for a co-pollutant. Similarly, there were no changes in the statistical significance and direction for the associations of 2 w O₃ exposure with IL-1 β , IL-2, IL-6, IL-17A, and IFN- γ , after controlling for a co-pollutant. However, the associations of the biomarkers with 24 h and 1 w O₃ exposure were changed to be non-significant after controlling for NO₂ as the co-pollutant.

The results from the two-pollutant models for the relationships between the biomarkers and NO₂ are shown in Figure C2. We found that the associations of 12 h NO₂ exposure with IL-1 β , IL-2, IL-6, and IL-10 and the associations of 24 h NO₂ exposure with IL-6 and IL-10 were not noticeably changed in terms of statistical significance and effect sizes after controlling for a second pollutant. However, the significant and positive association of the biomarkers and 1 w and 2 w NO₂ exposure were all changed to be non-significant after controlling for a second pollutant.

The results of two-pollutant models for the relationships of the biomarkers with PM_{2.5} and SO₂ are shown in Figure C3-C4. We found that all the associations were changed to be non-significant after controlling for a second pollutant.

4.1.3.5 Intervention effect model results

The associations of the ESP removal and biomarker concentrations are shown in Figure 12. We found that the removal of ESP for 17 to 38 days was associated with significant changes in multiple proinflammatory cytokines by -64.7% (-85.0% to -16.8%), -57.5% (-79.1 to -13.6%), -72.4% (-86.7% to -42.2%), -73.6% (-86.7% to -47.7%), -51.4% (-74.7% to -6.6%), and -61.4% (-80.1% to -25.5%) for IL-1 β , IL-2, IL-6, IL-8, IL-17A, and TNF- α , respectively.

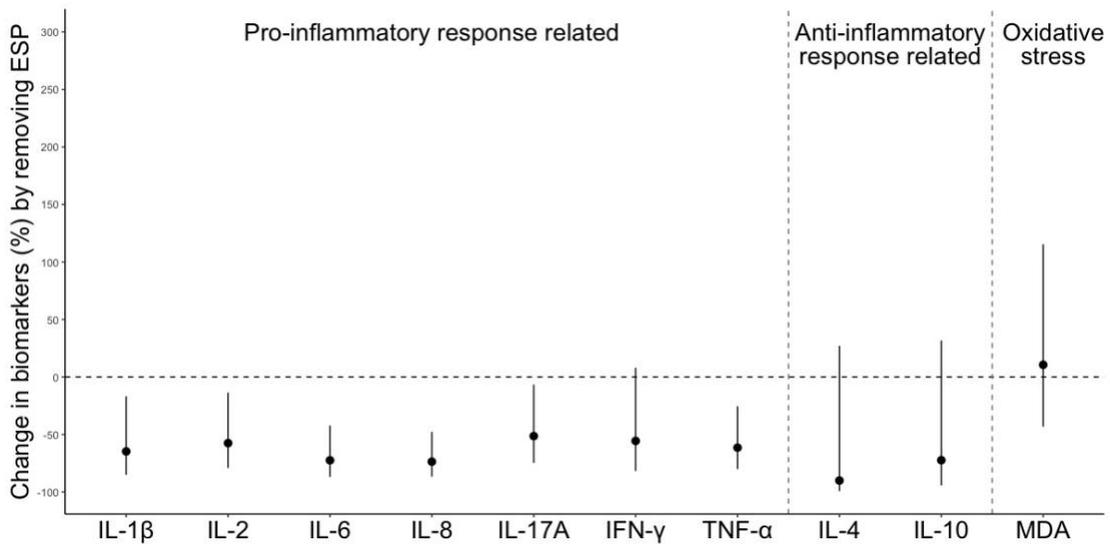


Figure 12. Mean change in biomarkers (%) and 95% CIs associated with the ESP removal from the ventilation system.

4.1.3.6 Sensitivity analysis

The exposure-response relationships were examined by excluding current smokers or subjects undergoing respiratory infection during the 1 week before the biospecimen collection. There were no noticeable changes compared to the main

findings on the O₃ exposure-response relationships, supporting the robustness of the main analysis (see Figure C5-C6).

4.1.4 Discussion

By removing an ESP in the air handling units for offices and residences of the study participants, we manipulated the personal O₃ exposure (see Table 7). In this study, we found that an increase in 12 h O₃ exposure was associated with decreased concentrations of two proinflammatory cytokines, IL-17A and IFN- γ , and the associations were not noticeably changed after controlling for a co-pollutant (see Figure 11). A potential mechanism is that exposure to O₃ for 12 h might stimulate the production of antioxidant enzymes as a compensatory mechanism.¹⁵⁶ The upregulation of antioxidant enzymes would reduce oxidative stress and might be associated with the suppression of proinflammatory responses.¹⁵⁷ This mechanism could be partially supported by the non-significant negative association of 12 h O₃ exposure and urinary MDA examined in this study. On the other hand, it was also reported that exposure to high-level O₃ (2.5 ppm) for 3 hours would induce mitochondrial dysfunction and activate the nucleotide-binding domain and leucine-rich repeat protein 3 (NLRP3) inflammasome, which plays a critical role in the pathogenesis of O₃-induced airway inflammation.¹⁵⁸ Future studies are needed to investigate the underlying biological mechanisms of both the pro- and anti-inflammatory responses to short-term (< 24 hours) O₃ exposure at different concentrations.

In contrast, we found that an increase in 2-week O₃ exposure was associated with increased concentrations of the proinflammatory cytokines (i.e. IL-1 β , IL-2, IL-6, IL-17A, and IFN- γ) from both the single- and two-pollutant models (see Figures 10 and 11). The results were supported by previous studies finding that chronic exposure to ozone was associated with adverse systemic inflammatory effects.¹⁵⁹ A potential explanation is that the persistent exposure to O₃ over a longer period might lead to the upregulation of internal redox homeostasis leading to an increased level in systematic inflammation.¹⁶⁰ Although in the current study, we did not find a significant effect of the 2 w average O₃ exposure on MDA concentration, this hypothesis could be supported by many previous studies finding that O₃ exposure averaged over 2-4 weeks prior were associated with the increased oxidative stress and systematic inflammation.¹⁶¹⁻¹⁶⁴ On the other hand, we found that the significant associations of 1 w O₃ exposure with the biomarkers were all changed to be non-significant in two-pollutant models. The results were not in line with a previous study reporting that exposure to O₃ for 1 week was associated with increased blood levels of tumor necrosis factor receptor 2, a proinflammatory cytokine.¹⁶⁵ The inconsistency might be attributed to different study designs, the subject demographic characteristics, confounders, and exposure inaccuracy.

In this study, we found that the removal of an ESP from the AHUs for 17 to 38 days contributed to the reduction in personal O₃ exposure, and this intervention was associated with decreased concentrations of all of the seven proinflammatory cytokines

measured in the current study (see Figure 12). This is consistent with our findings on the associations between inflammatory cytokines and 2 w average O₃ exposure. This finding is also supported by a previous study showing that reduced O₃ levels during the 2014 Nanjing Youth Olympics period of 5-week were associated with reduced levels of IL-1 β in healthy adults.¹⁶⁶ ESPs have been widely used to remove particulate matters but can produce O₃ as a by-product. The increase in O₃ exposure might have adverse health effects, which is a significant concern of this technology. Continued efforts should be made to minimize the O₃ production if an ESP is used in the presence of occupants.

In the present study, the personal PM_{2.5}, NO₂, and SO₂ exposures generally showed opposite associations with the biomarkers compared with O₃, especially NO₂ (see Figure 10). It might be due to the strong negative correlations of personal O₃ exposure with PM_{2.5}, NO₂ and SO₂ exposure (see Figure 9). We found that short-term (12 h or 24 h) NO₂ exposures were significantly associated with increased IL-1 β , IL-2, IL-6, IL-8, IL-17A, IFN- γ , and IL-10 in single pollutant models (see Figure 10). These associations were not noticeably changed after controlling for a second pollutant (see Figure C2). The results were partially supported by previous studies finding that exposure to NO₂ for 24 hours was associated with increased levels of IL-6.¹⁶⁷ Concerning NO₂ exposure is indicative for traffic-related pollutant exposures, including metals, ultrafine particles, and elemental carbon, the significant associations of NO₂ exposure

with inflammatory cytokines might be confounded by other traffic-related pollutants.¹⁶⁸

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Based on two-pollutant models, the associations of inflammatory cytokines with PM_{2.5} and SO₂ exposures were not found to be significant (see Figure C3). The results were partially supported by a previous study finding that exposure to PM_{2.5} at the level of 9.7-11 µg/m³ for 24h was not significantly associated with TNF-α and IL-6,^{123, 167} and an overnight (12 h) reduction on PM_{2.5} exposure was not found to be associated with IL-6.⁷¹ However, other studies also reported that short-term exposure to PM_{2.5} or to SO₂ were associated with increased cardiovascular mortality^{170, 171} and increased proinflammatory responses.¹⁷² Similarly, the unclear associations of 2 w averaged PM_{2.5} exposure with the biomarkers were inconsistent with many previous studies finding that PM_{2.5} exposure was associated with the upregulation of inflammatory cytokines.¹⁷³ The discrepancy might be due to different subject demographic characteristics, exposure inaccuracy, and potential confounders.

The results of sensitivity analysis did not markedly change the observed associations, supporting the robustness of the main analysis. However, this study is limited in controlling for other confounders that might have changed during the study period, including alcohol intake, medicine usage, and unmeasured pollutant exposures, etc.

4.1.5 Conclusion

Results from the association analyses suggest that exposures to air pollutants for different time durations affect systemic inflammatory and oxidative stress responses differently. A longer-term (2-week) exposure to O₃ was associated with increased concentrations of proinflammatory cytokines in the blood, consistent with the previous evidence that persistent exposure to O₃ can upregulate the redox homeostasis towards an enhanced proinflammatory status. In contrast, a shorter-term (12-hour) exposure O₃ was associated with decreased concentrations of proinflammatory cytokines, suggesting that O₃ may acutely stimulate the production of antioxidant enzymes as a compensatory mechanism. In addition, the significant associations between NO₂ exposures within a day (12-hour and 24-hour) and proinflammatory cytokines may be related to the effects of short-term exposure to traffic related pollutants on systemic inflammation. These mechanistic insights can be useful in developing preventive and therapeutic strategies to reduce the adverse effects of air pollution. Towards this goal, future research is recommended to examine the biological responses to a wider range of air pollutants and/or in more susceptible individuals (e.g., persons with asthma or other preexisting diseases).

4.2. Part B: Endogenous melatonin mediation of systemic inflammatory responses to ozone exposure in healthy adults

Part B is adapted with permission from He, L.; Hu, X.; Gong, J., Day, D. B.; Xiang, J.; Mo, J.; Zhang, Y.; Zhang, J.; Endogenous melatonin mediation of systemic inflammatory responses to ozone exposure in healthy adults. *Science of The Total Environment*. 2020 (Publisher: Elsevier). The accompanying supporting information is included in Appendix D. In this study and the published manuscript, I proposed the research idea, analyzed the urine samples for aMT6s, conducted the statistical analysis, interpretate the results, design all tables and figures, and led the writing and revising of the manuscript. The coauthors contributed to the study design (JG and JZ), manuscript revisions (DD and JZ), sample and data collection in the original study (YZ, JM, DD, and JX), sample analysis (HX), and discussions (JZ and DD).

4.2.1 Introduction

The levels of air pollutants, including ozone (O₃), fine particulate matter (PM_{2.5}), nitrogen dioxide (NO₂), and sulfur dioxide (SO₂), are elevated in urban air due to the presence of industrial and traffic emissions.²⁴ As an important risk factor to human health, air pollutant exposures have been associated with cardiopulmonary disease morbidity and mortality.^{133, 174}

Air pollutants can react with biomolecules to generate reactive oxygen species (ROS) and increase systemic oxidative stress, which may further trigger cardiorespiratory inflammatory responses.^{137, 138} This has been established as one of the

common pathophysiologic pathways linking air pollutant exposures with cardiopulmonary diseases.^{136, 163} Cytokines are major mediators of intercellular communication required for an integrated response to a variety of stimuli during immune and inflammatory processes.⁶⁰ Our group previously observed positive associations of 2-week O₃ exposure with pro-inflammatory cytokines, including interleukin 1 β (IL-1 β), interleukin 2 (IL-2), interleukin 17A (IL-17A), and interferon-gamma (IFN- γ).¹⁷⁵ In addition, it has been reported in previous studies that air pollutant exposures were associated with increases in pro-inflammatory cytokines, including IL-1 α/β , interleukin 6 (IL-6), interleukin 8 (IL-8), and tumor necrosis factor- α (TNF- α).^{16, 176,}

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Melatonin is a hormone excreted by the pineal gland with a marked circadian rhythm.³⁷ It is a widespread anti-inflammatory molecule, affecting inflammatory responses through various pathophysiological pathways.⁵⁸ For example, melatonin can modulate the nuclear factor kappa B (NF- κ B) signaling pathway, which plays a critical role in the regulation of a variety of important genes involved in cellular inflammatory responses.^{58, 60} In addition, melatonin can modulate the inflammasome (NLRP3), which responds to various damage-associated molecular signals.⁵⁸ The activation of NLRP3 can protect against invading pathogens, while its excessive activation can lead to tissue injury.⁵⁹ Taking melatonin supplementation was previously reported to suppress systemic inflammation;⁶³ however, the relationship between endogenously generated

melatonin and inflammatory responses is still unclear. Here, we hypothesize that natural levels of melatonin (i.e, without a supplementation) are negatively associated with concentrations of pro-inflammatory cytokines.

Melatonin is also a broad-spectrum antioxidant and free radical scavenger.¹⁷⁸ Circulating levels of melatonin and ROS could be affected by each other.⁸⁸ As it has been widely reported that air pollutant exposures are able to increase systematic oxidative stress, we hypothesize that increased air pollutant exposures are associated with decreased melatonin levels. Taking these two hypotheses together, we further hypothesize that melatonin can mediate inflammatory responses to pollutant exposures.

To test these hypotheses, we analyzed the stored biospecimens collected from a previous study that originally investigated the effects of an indoor air quality intervention on cardiorespiratory health outcomes.^{14, 147} We measured 9 inflammatory cytokines in plasma and urinary 6-sulfatoxymelatonin (aMT6s), a major metabolite of melatonin, as a surrogate measure of circulating melatonin.⁴⁰ We aim to investigate (1) the relationships of aMT6s with air pollutant exposures, (2) the relationships of inflammatory cytokines with aMT6s, and (3) whether aMT6s is a mediator for the relationships between pollutant exposures and inflammatory cytokines.

4.2.2 Methods

4.2.2.1 Study participants and biospecimen collection

The current study used the data collected from an existing interventional study, and the detail study design has been previously reported.^{14, 147, 175} Below we describe the information pertinent to the present study. From December 6th, 2014 to January 13th, 2015, fifty-three healthy office workers, 22-52 years old, were recruited from a company campus in Changsha City, Hunan, China. All the subjects were free from chronic diseases and include self-reported current smokers. In the present analysis, we aim to examine the relationships of aMT6s with air pollutant exposures and inflammatory cytokines and to investigate whether aMT6s is a mediator for the pollutant exposures-cytokines relationships. The analysis is based on a panel study design as both early morning (7am – 9am) urine and fasting blood were collected from each participant three times, with about two weeks in between each two consecutive collections. All the biospecimens were aliquoted in sterilized plastic tubes and stored at -80°C before analysis. The study protocol was approved by the Ethics Committee of Shanghai First People's Hospital and the Duke University Campus Institutional Review Board.

4.2.2.2 Measurements of aMT6s and inflammatory cytokines

Urinary aMT6s was analyzed using a high pressure liquid chromatography-tandem mass spectrometry (HPLC-MS-MS) method.¹²¹ Concentrations of urinary aMT6s were normalized by creatinine to correct urine dilution. Urinary creatinine was

measured using a Creatinine (urinary) Colorimetric Assay Kit (Cayman Chemical, item No. 500701) and a microplate reader. We analyzed nine inflammatory cytokines in plasma using a commercially available analyzing kit (Human Cytokine/Chemokine Magnetic Bead Panel, Millipore Corporation, MA, USA), and the detailed protocol was published previously.¹⁷⁵ Among these cytokines, seven are related to pro-inflammatory responses, including IL-1 β , IL-2, IL-6, IL-8, IL-17A, IFN- γ , and TNF- α ,¹⁷⁹ and two are related to anti-inflammatory response, including interleukin 4 (IL-4) and interleukin 10 (IL-10).¹⁸⁰

4.2.2.3 Air pollutant exposure assessment

Indoor O₃ and PM_{2.5} concentrations were continuously monitored in the offices and dormitory rooms where participants were living and working on campus. Ambient PM_{2.5}, O₃, NO₂, SO₂, temperature, and relative humidity were monitored at a government station (~4.5 km from the company campus). Estimated indoor/outdoor (I/O) ratios of 0.35 for O₃ and 0.8 for PM_{2.5} were used to estimate indoor pollutant concentrations in unknown microenvironments (other than company dorms and offices) according to previous literature on lightly sealed buildings.¹⁴⁸⁻¹⁵⁰ We used 0.8 and 0.5 as the I/O ratios for NO₂ and SO₂, respectively, to calculate all indoor micro-environments, based on previous findings in dorms and buildings with similar physical characteristics.^{151, 152} These pollutant measurements were combined with detailed time-activity data collected from each participant to calculate personal air pollutant exposure

averaged over 12-hour (12h), 24-hour (24h), 1-week (1w), and 2-week (2w) periods prior to biospecimen collections. The time-activity data were obtained by asking participants to fill a questionnaire at each visit for biospecimen collection. The time-activity questionnaire was consisted of three following sections. The first section asks about the personal conditions, including whether the subject had a respiratory infection or had sudden medication changes before each visit. The second section recorded total times (hours) that participants spent in their offices, dormitories, and other places during the past 7 days. The specification of the other locations and how many hours were spent in each of other locations were recorded. The third section included the most detailed information, asking over the past 24 hours which specific times (down to the minute) the participant had spent in their offices, dormitories, outside the company campus and where those locations were, inside in other environments and where those locations were. The detailed description of the exposure assessment has been previously published.¹⁴

4.2.2.4 Statistical analysis

Based on data distributions, air pollutant exposure and concentrations of aMT6s and all the inflammatory cytokines were natural logarithm-transformed. The spearman correlations were examined among personal pollutant exposures.

First, we used linear mixed-effects regression (LMER) models to assess the relationships of aMT6s with personal pollutant exposures. In this set of models, aMT6s

concentration was regressed on each of the personal air pollutant exposures averaged over 12h, 24h, 1w, and 2w prior to biospecimen collection, controlling for ambient relative humidity and temperature averaged over the same time period as the pollutant exposure, sex, age, body mass index (BMI), upper respiratory infection status, and smoking status. Subject ID and the day of week for biospecimen collection were treated as the random intercepts in this and all subsequent LMER models. These covariates have been associated with systemic oxidative stress and inflammation in previous studies ⁷¹.

Second, we used the LMER models to examine the associations of aMT6s with each of the inflammatory cytokines individually. These models included the aforementioned covariates with the addition of a categorical variable for cytokine analysis batch to adjust for potential batch effects on cytokine measurements.

Third, we selected the cytokines and air pollutant exposures with significant coefficients in the previous analyses and conducted a mediation analysis using both mediator models and outcome models. The selection criteria included (1) having significant pollutant-cytokine association independent of co-pollutant exposure examined in our previous study,¹⁷⁵ (2) having significant pollutant-aMT6s association, and (3) having significant aMT6s-cytokine association. The mediator models were LMERS in which the aMT6s concentration was regressed on a given air pollutant exposure measure with the same covariates and random effects used in the first set of models. Similarly, LMERS were also used for the outcome models, in which each of the

cytokine concentrations was regressed on a given air pollutant exposure measure, aMT6s, and the covariates described in the second set of models. The mediation analysis used quasi-Bayesian approximation with a simulation of 5,000 iterations, which has been suggested by a previous study to ensure stable estimation.¹⁸¹ This analysis estimates the total effects (the total effect of pollutant exposure on cytokines), average direct effects (ADE, the average direct effect of pollutant exposure on cytokines), and average causal mediation effects (ACME, the average causal mediation effect of pollutant exposure on cytokines mediated by melatonin).

Finally, we conducted two sensitivity analyses: (1) We excluded measurements from current smokers or measurements from subjects who reported having an upper respiratory infection. (2) We used co-pollutant models to examine whether the relationships obtained in the single-pollutant models changed after controlling for a co-pollutant. (3) We included ambient temperature averaged over 12-hour, 24-hour, 1-week, and 2-week all into our models to examine the prolonged effects of temperature. In our statistical models, socioeconomic status (SES) was not considered as covariates given that all the study participants worked on similar jobs in the same company. All statistical analyses were conducted using R software (version 3.6.1) with the *lme4*, *lmeTest*, and *mediation* packages.¹⁸²⁻¹⁸⁴ A p-value of 0.05 was used as the cutoff for statistical significance. A detailed description of equations and codes for the statistical

models is provided in the Supplemental Material. The detailed model results (effect sizes, CIs, and p-values) are shown in Table D1-D2.

4.2.3 Results

4.2.3.1 Subject characteristics

Characteristics of the study subjects are summarized in Table 9. Of the 53 subjects, 16 (30.2%) were female and 11 (20.8%) were current smokers. The mean (SD) age of the subjects is 31.4 (7.3) year. A total of 145 measurements were obtained from all subjects, and 13 measurements of them reported respiratory infection during the 1-week prior to biospecimen collection.

Table 9. Characteristics of study subjects.

Subject Characteristics	Value
Female, No. (%)	16 (30.2%)
Age, years, mean \pm SD (range)	31.4 \pm 7.3 (22 - 52)
Current Smoker, No. (%)	11 (20.8%)
Respiratory infection, No. (%)	13 (9.0%)
BMI, Kg/m ² , mean \pm SD (range)	22.5 \pm 2.6 (18.4 - 29.4)

Table 10. Personal pollutant exposures to PM_{2.5}, O₃, NO₂, and SO₂ averaged over the 12-hour, 24-hour, 1-week, 2-week period prior to biospecimen collection.

		Mean ± SD	Median (IQR)	Range
O ₃ (ppb)	12h	6.8 (4.4)	5.0 (7.4)	1.4 – 21.6
	24h	8.0 (4.7)	5.8 (8.4)	1.7 – 19.3
	1w	8.9 (3.5)	6.9 (7.1)	4.7 – 15.9
	2w	8.7 (2.2)	8.1 (4.2)	5.5 – 13.2
PM _{2.5} (µg/m ³)	12h	38.6 (32.1)	28.2 (35.0)	3.7 – 169.1
	24h	32.8 (22.8)	26.8 (26.6)	4.5 – 112.2
	1w	30.6 (15.0)	27.7 (23.4)	11.2 – 79.9
	2w	36.6 (10.9)	35.5 (15.0)	13.0 – 71.3
NO ₂ (ppb)	12h	25.9 (6.4)	24.0 (6.3)	16.2 – 41.5
	24h	25.9 (5.5)	25.8 (5.2)	17.7 – 39.2
	1w	24.3 (5.6)	25.0 (9.6)	16.6 – 35.2
	2w	25.8 (2.9)	24.9 (3.6)	20.6 – 31.2
SO ₂ (ppb)	12h	6.2 (1.7)	5.7 (2.4)	3.7 – 11.8
	24h	6.7 (1.8)	6.3 (3.1)	3.6 – 10.6
	1w	6.0 (1.1)	6.0 (1.7)	4.5 – 8.5
	2w	5.8 (1.1)	5.8 (2.5)	4.2 – 7.8

4.2.3.2 Air pollutant exposure

The personal air pollutant exposures averaged over 12h, 24h, 1w, and 2w prior to each of visits for biospecimen collections are summarized in Table 10. Spearman correlations between pollutant exposure measures are shown in Figure 13. There were strong negative correlations between 2w O₃ exposure and both 2w NO₂ and SO₂ exposures. We also found strong positive correlations among 1w PM_{2.5}, NO₂, and SO₂ exposures.

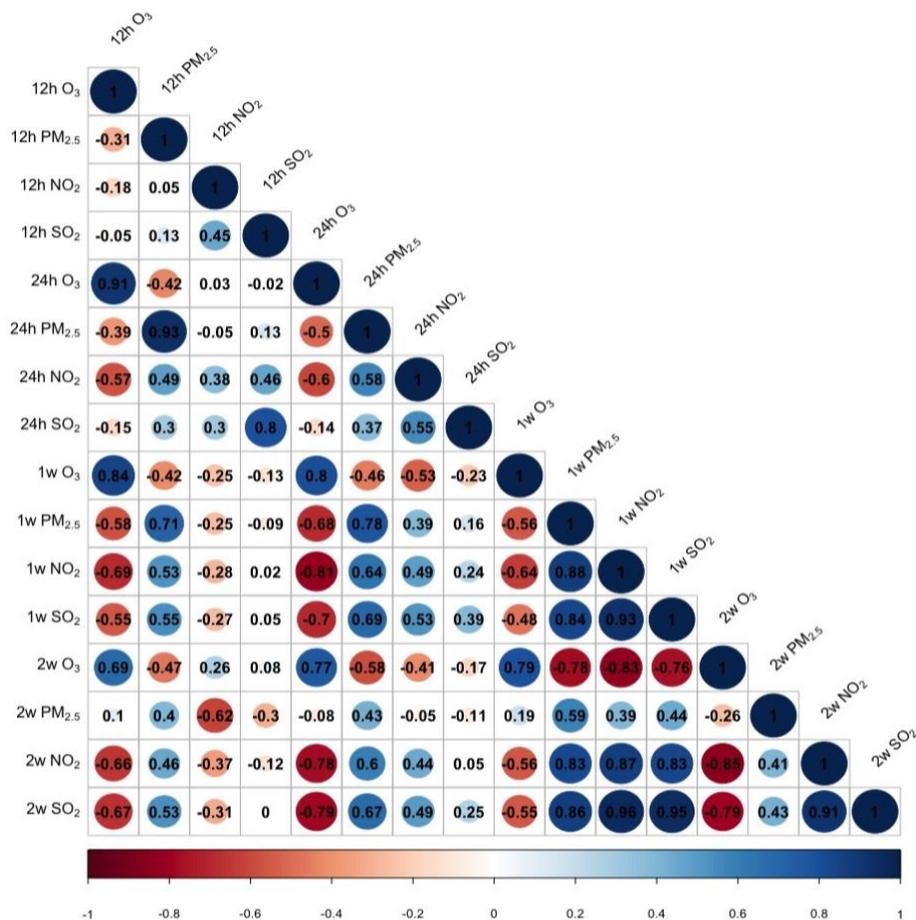


Figure 13. Spearman correlation coefficients among personal air pollutants exposure.

4.2.3.3 Relationships of aMT6s with pollutant exposures

The concentrations of urinary aMT6s and the plasma cytokines are shown in Table 11. The associations between air pollutant exposures and aMT6s are shown in Figure 14. We found that an IQR increase in 2-week O₃ exposure was significantly associated with a -26.2% (-43.9% to -2.8%) decrease in aMT6s. In contrast, IQR increases in 2-week PM_{2.5} and SO₂ were associated with 20.1% (1.7% – 41.8%) and 59.4 % (14.8% –

121.4%) increases in aMT6s, respectively. There were no significant associations between aMT6s and NO₂ exposure.

Table 11. Concentrations of urinary aMT6s and plasma inflammatory cytokines.

	Mean ± SD	Median (IQR)	Range
aMT6s (ng/mg creatinine)	11.2 ± 9.1	8.4 (10.3)	0.5 – 53.0
IL-1β (pg/mL)	2.7 ± 2.2	1.9 (2.2)	0.3 – 12.6
IL-2 (pg/mL)	2.8 ± 2.4	2.3 (1.9)	0.5 – 22.5
IL-6 (pg/mL)	1.1 ± 0.8	0.8 (0.8)	0.12 – 3.8
IL-8 (pg/mL)	3.1 ± 3.9	2.0 (2.2)	0.1 – 31.6
IL-17A (pg/mL)	5.5 ± 5.6	3.5 (4.0)	0.7 – 31.2
IFN-γ (pg/mL)	17.6 ± 17.5	12.9 (19.8)	0.8 – 100.3
TNF-α (pg/mL)	12.5 ± 5.7	11.2 (9.1)	0.5 – 28.2
IL-4 (pg/mL)	16.9 ± 27.5	4.7 (18.7)	0.01 – 145.5
IL-10 (pg/mL)	6.8 ± 11.6	2.5 (6.9)	0.02 – 87.9

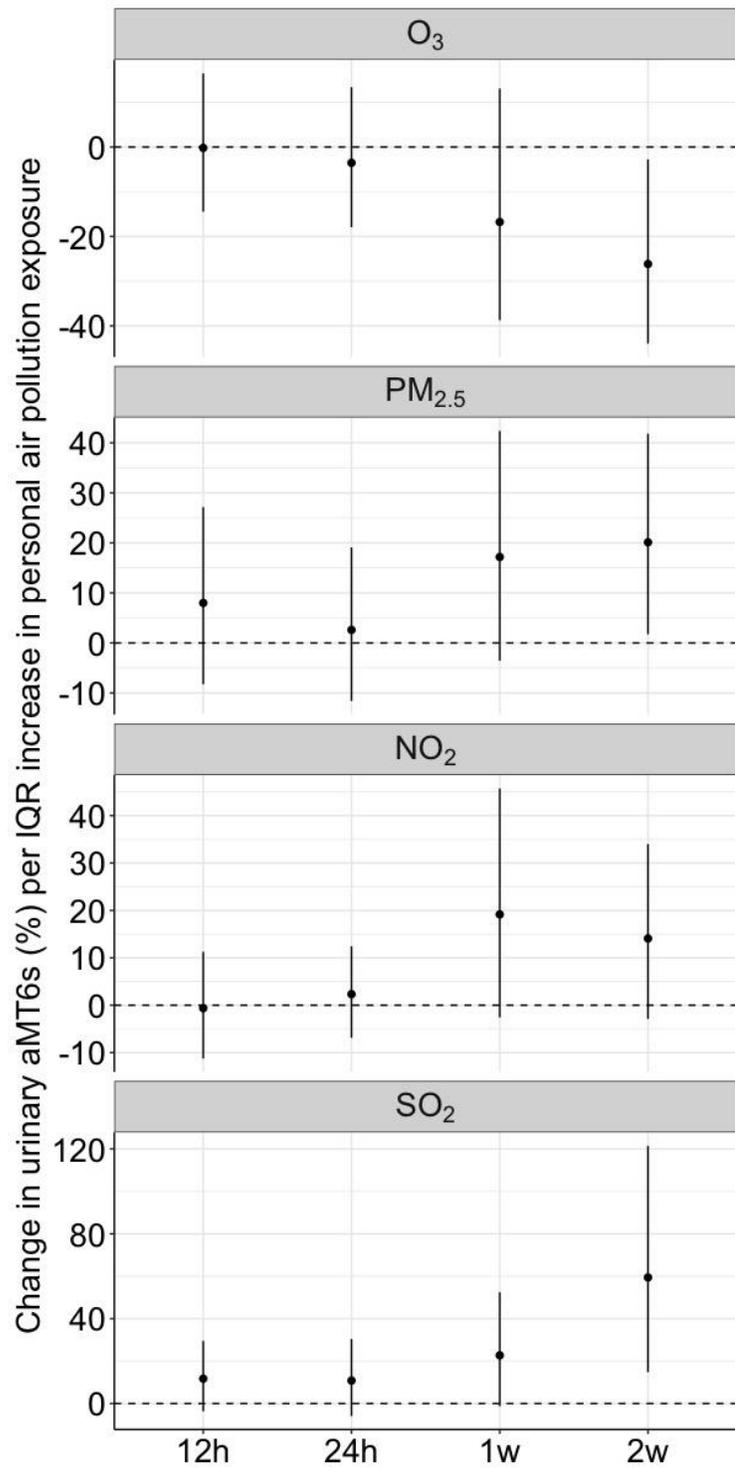


Figure 14. Estimated means and 95% CIs for change in urinary aMT6s (%) per IQR increase in 12h, 24h, 1w, and 2w pollutant exposure.

4.2.3.4 Relationships between aMT6s and inflammatory cytokines

The associations between aMT6s and inflammatory cytokines are shown in Figure 15. We found that an IQR increase in aMT6s was associated with decreases in pro-inflammatory cytokines, including IL-1 β , IL-8, IL-17A, IFN- γ , and TNF- α by 17.8% (-31.4% to -1.6%), 21.6% (-35.2% to -5.2%), 18.8% (-32.0% to -3.0%), 27.2% (-42.0% to -8.7%), and 17.6% (-28.2% to -5.6%), respectively. In contrast, the associations between aMT6s and the anti-inflammatory cytokines, IL-4 and IL-10, were nonsignificant.

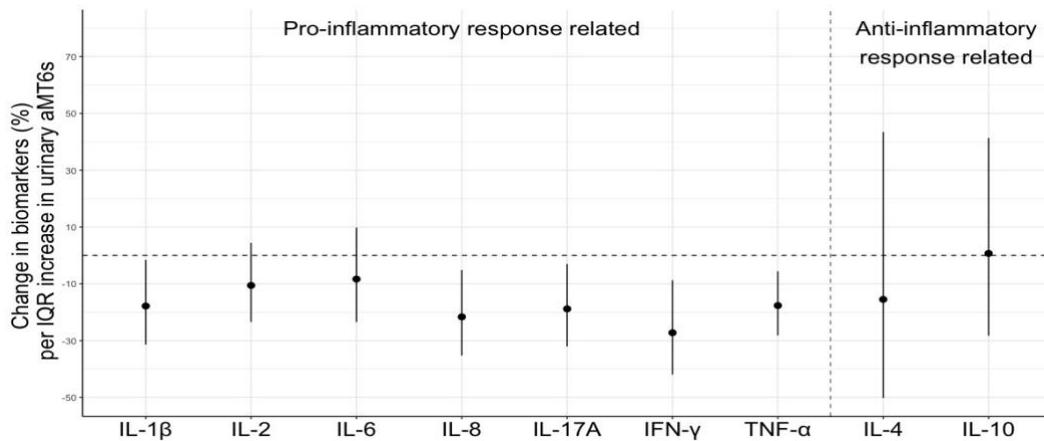


Figure 15. Estimated means and 95% CIs for change in inflammatory cytokines (%) per IQR increase in aMT6s.

4.2.3.5 Mediation analysis

In our previous study, we only found significant associations of 2w O₃ with the pro-inflammatory cytokines independent from co-pollutant exposures.¹⁷⁵ In this study, we further found significant associations of aMT6s with 2w O₃ exposure and some of the pro-inflammatory cytokines, including IL-1 β , IL-8, IL-17A, IFN- γ , and TNF- α . Hence, the mediation analyses were conducted to test whether aMT6s mediated the

relationships of 2w O₃ exposure with IL-1 β , IL-8, IL-17A, IFN- γ , and TNF- α . The total effects, ADE (average direct effect), and ACME (average causal mediation effect) of 2w O₃ exposure on inflammatory cytokines are shown in Figure 16. We found significant and positive total effects of 2w O₃ exposure on all the five pro-inflammatory cytokines. However, by excluding the effects of aMT6s on the cytokines, the direct effects (ADE) of 2w O₃ exposure on these cytokines were diminished compared to their total effects. The difference between the total effects and ADE, shown as ACME, indicate the casual mediation effects of aMT6s on the 2w O₃ exposure-cytokines relationships.

Specifically, the proportions of the 2w O₃ exposure-cytokines relationships mediated by aMT6s were shown in Table 12. We found that 12.1% (0.49% – 53.8%), 7.4% (-1.3% – 24.1%), 8.3% (-0.59% – 28.0%), 11.3% (1.1% – 32.0%), and 17.4% (0.91% – 82.7%) of the total effects of 2w O₃ exposure on IL-1 β , IL-8, IL-17A, IFN- γ , and TNF- α were mediated by aMT6s.

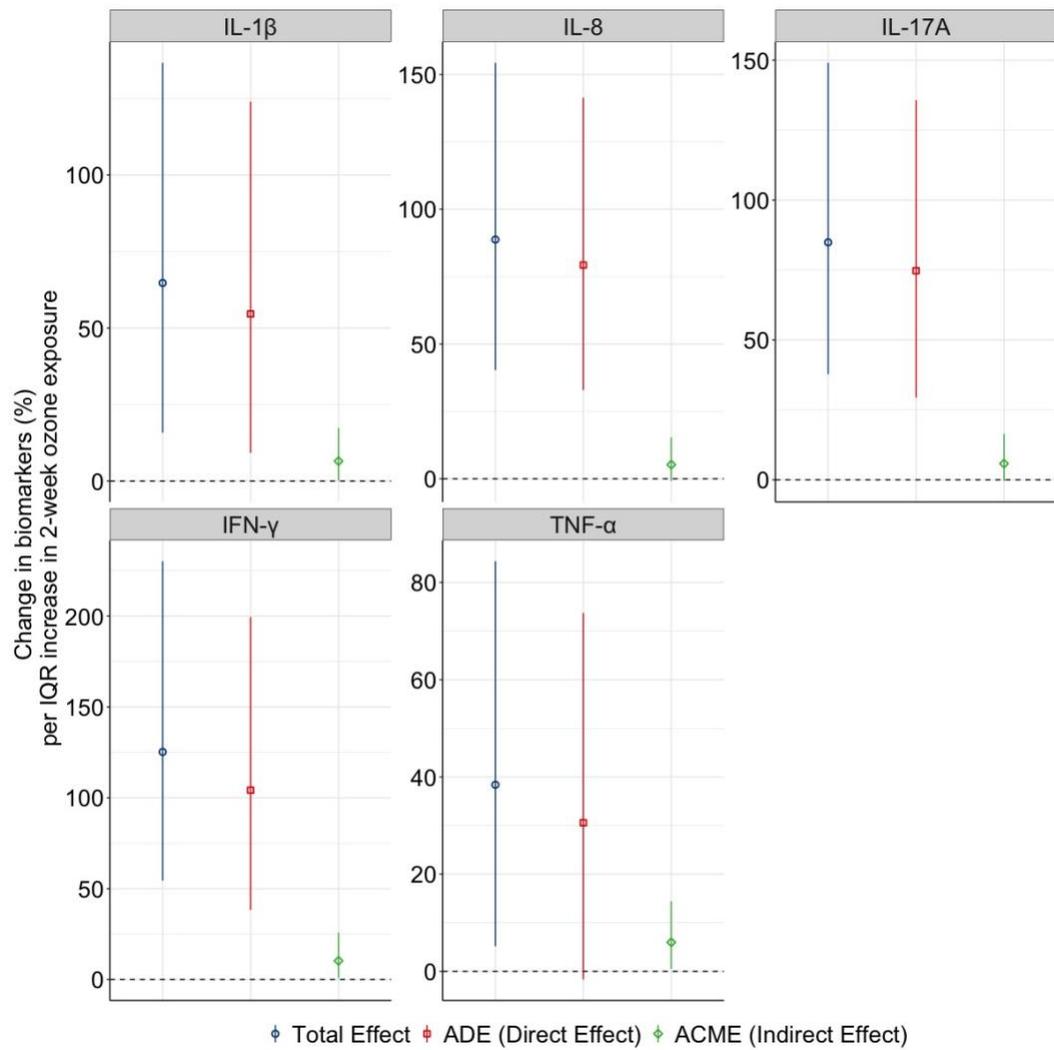


Figure 16. Change in cytokine concentration associated with one IQR increase in 2-week O₃ exposure for total effect, ADE, and ACME, respectively.

Table 12. The proportion of the total effects of 2-week O₃ exposure on the five pro-inflammatory cytokines mediated by aMT6s.

	Proportion (%)	95% CI (%)	P-value
IL-1 β	12.1	(0.49 – 53.8)	0.044
IL-8	7.4	(-1.3 – 24.1)	0.11
IL-17A	8.3	(-0.59 – 28.0)	0.09
IFN- γ	11.3	(1.1 – 32.0)	0.025
TNF- α	17.4	(0.91 – 82.7)	0.036

4.2.3.6 Sensitivity analyses

First, we conducted sensitivity analyses using datasets excluding current smokers or measurements from participants who reported respiratory infection 1-week prior to biospecimen collection. In these analyses we found no substantial differences in the associations between aMT6s and cytokines in terms of both statistical significance and effect sizes (Figure D1 and D3). In addition, there were no noticeable differences in the effect sizes of relationships of air pollutant exposures with aMT6s; except for the association of 2w O₃ exposure with aMT6s was changed to be nonsignificant (Figure D2 and D4). Similarly, the results of mediation analysis were not remarkably changed after excluding measurements for which participants reported respiratory infection (Table D3). However, we found that the mediation effects of aMT6s on the association of 2w O₃ exposure with IL-1 β and TNF- α became nonsignificant when excluding current smokers, while the effect sizes were increased (Table D4). Second, the results of two pollutant models show that the significant associations of aMT6s with 2w O₃ and SO₂ exposure was changed to be nonsignificant after controlling for a co-pollutant exposure,

while the effect size was not substantially changed (Figure D5). However, the associations of 2w PM_{2.5} and aMT6s became not only nonsignificant but also substantially decreased in effect size after controlling SO₂ or O₃ as a co-pollutant exposure. Third, after controlling for all the four temperature measurements (i.e. averaged over 12h, 24h, 1w, and 2w) in our models, we did not observe remarkable changes in the relationship of 2-week pollutant exposures with aMT6s, in terms of both effect sizes and statistical significance (Figure D6).

4.2.4 Discussion

The main findings of this study are that urinary concentrations of aMT6s are negatively associated with both 2w O₃ exposure and the pro-inflammatory cytokines. In addition, mediation analyses suggest that urinary aMT6s is a potential mediator for the relationships between 2w O₃ exposure and the pro-inflammatory cytokines, including IL-1 β , IFN- γ , and TNF- α . In the current study, none of the subjects reported taking melatonin supplementation. This was supported by the range of urinary aMT6s concentration measured in this study, corresponding to 0.5 to 53.0 ng/mg creatinine, which was within the range previously measured for people without taking exogenous melatonin.¹⁰⁷ Our results suggest that the inflammatory effects of ozone are partially mediated through the reduction of endogenous melatonin, and typical endogenous concentrations of melatonin may be sufficient to moderate the inflammatory effects of ozone exposure.

In this study, we did not find any significant associations between aMT6s and any pollutant exposure averaged over 12h or 24h. These results are consistent with a previous study reporting that short-term (12h and 24h) PM_{2.5}, O₃, and NO₂ exposure were not associated with significant changes in urinary aMT6s.¹⁵⁴ A potential explanation is that oxidative stress induced by short-term air pollution exposure might be neutralized by pre-existing antioxidants in the lung, leading to a suppressed effect on endogenous circulating melatonin level. In contrast, we found that increased 2w O₃ exposure was associated with decreased aMT6s concentration. It has been widely reported that O₃ exposure leads to increased oxidative stress in the respiratory tract, and this may further spill over to circulatory system.¹⁸⁵ This result could be also supported by our previously findings in the same study subjects that an increase in 2-week low-level O₃ exposure was significantly associated with increased 8-hydroxydeoxyguanosine (8-OHdG), a stable product of DNA oxidation by ROS.¹⁴ Consistently, increased ROS production, through shifting the redox homeostasis towards a higher oxidation status due to longer-term low-level O₃ exposure, enhanced the consumption of melatonin as an antioxidant molecule and the oxidative damage to DNA.¹⁶⁰ However, shorter-term low-level exposure might not be sufficient to shift the redox homeostasis in the circulatory system. This hypothesis could be supported by a previous study reported that, compared with administrative staff, the long-term exposure of electronic equipment repairers to extremely low frequency electromagnetic fields known to produce ozone as

a byproduct had higher systemic oxidative stress levels and lower serum melatonin levels.¹⁸⁶

Exogenous melatonin has been reported to modulate inflammatory cytokines in both human and animals.⁵⁸ For example, a double blind, randomized, placebo-controlled trial reported that orally administration of 25 mg/d melatonin for 6 months was associated with significantly decreased serum concentration of pro-inflammatory cytokines in mice, including IL-1 β , IL-6, and TNF- α .⁶³ Another randomized trial found that receiving daytime administration of melatonin (100 mg) before endotoxemia reduced pro-inflammatory cytokines level in people (i.e. IL-1 β).⁶⁴ However, the role of endogenously generated melatonin in modulating inflammatory responses is still unclear. In this study, we found that, within the natural range of urinary aMT6s concentration, increased aMT6s concentration was associated with significantly decreased plasma pro-inflammatory cytokine levels. The result was supported by a previous study reporting that the increased excretion of melatonin during the night time was associated with decreased blood levels of IL-6 in patients with acute myocardial infarction.¹⁸⁷ On the other hand, the associations between aMT6s and anti-inflammatory cytokines were inconclusive and nonsignificant in the present study. The results could be indirectly supported by a previous study finding that no significant changes in anti-inflammatory cytokines were found in people receiving melatonin administration.⁶⁴ In contrast, another study reported that taking melatonin supplementation could

upregulate the expression of anti-inflammatory genes.¹⁸⁸ Further research should investigate the underlying biological mechanisms of how endogenously generated melatonin modulates both pro- and anti-inflammatory responses.

In this study, we found that the increase in 2w PM_{2.5} exposure was associated with a significant increase in aMT6s only in the single pollutant models. The positive association between PM_{2.5} exposure and aMT6s was in line with a previous study finding that increased 9-day average PM_{2.5} exposure was associated with increased serum melatonin levels in healthy adults.¹²³ The underlying biological mechanisms are still unclear. It has been recently reported that melatonin could be synthesized in mitochondria in response to increased cellular oxidative stress.¹²⁵ Hence, a potential mechanism is that PM_{2.5} exposure may increase cellular oxidative stress levels, which may consequently stimulate the production of melatonin in the cells. In addition, the negative association between 2w O₃ exposure and aMT6s led us to further hypothesize that O₃ exposure may not trigger or has limited effects on this stimulation process. On the other hand, we also found 2w SO₂ exposure was significantly and positively associated with aMT6s (see Figure 15). This result might be attributed to the strong negative correlation of 2w O₃ exposure with SO₂ (see Figure 14).

Smoking and respiratory infection may affect inflammatory responses and induce oxidative stress, affecting the circulating melatonin level. Therefore, we re-conducted the analyses using datasets excluding current smokers or measurements from

participants who reported respiratory infection. The results of sensitive analyses did not noticeably change the observed associations of aMT6s with the cytokines and air pollutant exposures in terms of effect sizes (see Figure D1-D4). However, the association of 2w O₃ exposure and aMT6s was changed to be nonsignificant. Similarly, the effect sizes of the associations examined in mediation analyses were not remarkably changed using the new datasets, except that the mediation effects of aMT6s on 2w O₃ exposure with IL-1 β and TNF- α became nonsignificant when excluding current smokers (see Table D3 and D4). These discrepancies might be attributed to the reduced sample size and statistical power when excluding 19% of participants. In addition, after controlling for a co-pollutant exposure, the associations of 2w O₃ and SO₂ exposure and aMT6s became nonsignificant, while the effect sizes remained similar (see Figure D5). This suggests that the coefficient estimates for these associations were not biased due to uncontrolled confounding by a co-pollutant, but rather that controlling for additional pollutants decreased estimate precision and our ability to detect this association, which perhaps could be remedied with a larger sample size. However, after controlling O₃ or SO₂ as a co-pollutant exposure, the association of 2w PM_{2.5} and aMT6s both decreased markedly in effect size and became nonsignificant, suggesting that O₃ and SO₂ confounded the association between PM_{2.5} on aMT6s. Additional research is needed to further characterize the effects of single air pollutant exposure on circulating melatonin level.

A limitation of this study is the lack of direct light exposure measurement, concerning that light/dark cycle dramatically affects circulating melatonin level. Another limitation is the lack of direct sleep condition measurement, concerning that sleep quantity and quality could affect both circulating melatonin and ROS level. However, the influence of these limitations on the findings was minimized by the following features of the study design and data analysis. (1) In considering the diurnal variation in circulating melatonin level, all urine and blood samples were collected during the early morning. (2) All the study subjects were working and living in a company campus with a relative uniformity of time/activity pattern (e.g. work and sleep schedule). (3) Ambient relative humidity and temperature, as the indicators of outdoor weather, were controlled in our models as a proxy for outdoor light exposure. (4) Linear mixed effects models were used with subject ID as the random effect to control for the potential differential average light exposure and sleep quality of different subjects. The current study is also limited by its relatively small sample size and by potential confounding. Future studies to confirm and expand the findings of this study should consider a design with a larger sample size, longer air pollutant exposure duration, well-controlled co-pollutant exposures, and/or well-controlled time/activity patterns.

4.2.5 Conclusion

Within the natural range of aMT6s concentrations, increased aMT6s was associated with decreased pro-inflammatory cytokine concentrations, suggesting that

endogenously-generated circulatory melatonin modulates pro-inflammatory responses. In addition, increased 2w O₃ exposure was associated with decreased aMT6s level; however, this association was potentially confounded by co-pollutant exposure. This study found that aMT6s is a potential mediator for the associations of 2w O₃ exposure with pro-inflammatory cytokines, which suggests that pro-inflammatory responses to O₃ exposure in the preceding 2 weeks may partly result from the depletion of endogenous melatonin by O₃.

Chapter 5. The role of melatonin in physiological and oxidative stress responses to air pollution exposure in asthmatic children

This chapter address Aim 4, in which urine and nasal fluid samples and pulmonary physiology outcomes were obtained from a previous panel study that investigated the pulmonary health effects of air purification intervention.⁷² In this Aim, urinary aMT6s and 8-OHdG and nasal fluid MDA were further measured and detailed personal air pollution exposure assessment was conducted. This chapter includes three parts: Part A, Part B, and Part C.

In Part A, the associations of personal air pollutant exposures with indicators of pulmonary physiology, including airway mechanics, lung function, airway inflammation, were examined. In Part B, the associations among air pollution exposure, nasal MDA, and asthma symptom scores were examined to investigate whether nasal MDA is useful for monitoring asthma symptoms and lung physiology in relation to air pollution exposure. In Part C, the associations of urinary aMT6s with systemic oxidative stress, airway mechanics, lung function, airway inflammation were examined. In addition, I investigated whether the associations between urinary aMT6s and the pulmonary physiology outcomes were confounded by systemic oxidative stress.

5.1 Part A: Associations of personal exposure to air pollutants with airway mechanics in children with asthma.

This Part A is adapted with permission from He, L.; Li, Z.; Teng, Y.; Cui, X.; Barkjohn, K. K.; Norris, C.; Fang, L.; Lin, L.; Wang, Q.; Zhou, X.; Hong, J.; Li, F.; Zhang, Y.; Schauer, J. J.; Black, M.; Bergin, M.; Zhang, J., Associations of personal exposure to air pollutants with airway mechanics in children with asthma. *Environment International*. 2020, 138 (Publisher: Elsevier). The accompanying supporting information is included in Appendix E. In this study and the published manuscript, I conducted the air pollution exposure assessment, conducted the statistical analysis, interpretate the results, design all tables and figures, and led the writing and revising of the manuscript. The coauthors contributed to the study design (JZ, ZL, JS, MB, and MHB), funding acquisition (JZ, MHB, JS, ZL, and YZ), manuscript revisions (JZ), sample and data collection (ZL, YT, XC, KB, CN, LF, LL, QW, XZ, JH, FL, YZ, JS, MHB, and JZ), sample analysis (XC and YT), and discussions (JZ).

5.1.1 Introduction

Airborne fine particles with an aerodynamic diameter $\leq 2.5\mu\text{m}$ (PM_{2.5}) and ozone (O₃) are well-established risk factors for asthma exacerbation.²² Elevated PM_{2.5} and O₃ levels are found in polluted urban atmospheres worldwide.²⁴ Indoor PM_{2.5} concentrations are substantially elevated in the presence of smoking, cooking, and floor vacuuming. Indoor concentrations are also elevated by the infiltration of outdoor PM_{2.5} and O₃ in poorly sealed building.^{189, 190} Hence, it is important to understand how

personal exposure to these pollutants can affect the physiology and function of the asthmatic lung.

Spirometric lung function, widely used in asthma diagnosis and prognosis, has been associated with personal air pollutant exposure. For example, a previous study reported that the increase in PM_{2.5} exposure measured 24-hour prior was associated with decreased forced expiratory volume in the first second (FEV₁) in children with asthma.²⁶ Another study reported that the increase in 24-hour average personal PM_{2.5} exposure measured one day prior was associated with a decrease in peak expiratory flow (PEF) in asthmatic children.²⁵ In contrast, a different study, also in asthmatic children, did not find significant associations between FEV₁ and PM_{2.5} exposure measured 24-hour prior.²⁷ The inconsistency has been attributed to differences in a variety of factors such as PM_{2.5} exposure level and/or range, demographic characteristics, air pollution composition, and other unmeasured potential confounders. In addition, lung function changes require a relatively strong acute stimulus or persistent stress to the lung.

Compared to lung function, airway mechanics are a more sensitive metric used to monitor airway obstruction and airflow limitation. Although clinical bench mark values are yet to be established, airway mechanics have been increasingly used as a supplement to spirometry for diagnosing asthma and to monitor disease prognosis.^{28, 29} In addition, while spirometry requires a subject to blow as hard as possible, impulse oscillometry (IOS) is conducted with normal breathing to measure airway mechanics.

This minimizes the data loss due to technical noncompliance from certain subjects, especially children, who cannot perform an accurate spirometry test.¹⁹¹ However, to the best of our knowledge, no studies have investigated the relationships of airway mechanics with personal air pollutant exposure in children with asthma.

We hypothesized that increasing personal air pollutant exposure is associated with worsening airway mechanics. To test this hypothesis, we used the data collected from a cohort of children with asthma. We aim to perform exposure-response analyses in examining the associations of airway mechanics (and lung function) with 24-hour average personal pollutant exposure measured zero to six days prior to outcome measurements in asthmatic children. As asthma is a heterogenous disease, we further explored whether baseline airway eosinophilic inflammation would affect the exposure-response relationships.¹⁹²

5.1.2 Methods

5.1.2.1 Study participants

We recruited 43 children (26 boys and 17 girls) 5 to 13 years old from a pool of children who had been diagnosed with mild or moderate asthma at the outpatient clinic of the Shanghai General Hospital, located in Songjiang, a suburb of Shanghai, China. In order to be eligible, participants should have physician-diagnosed asthma and at least one asthma attack during the past year. All the individuals with chronic diseases other than asthma were excluded. Oral assent and written consent were obtained from all

participants and their guardians, respectively. The Ethics Committee of Shanghai General Hospital and Duke University Campus IRB approved the study protocol.

5.1.2.2 Study design

The present study used data that had been collected in an indoor air filtration intervention in which indoor PM_{2.5} and O₃ concentrations were manipulated through operation of a portable air purifier (Atmosphere[®], Amway, USA) in participants' bedrooms.^{193, 194} Briefly, each air purifier was equipped with a coarse filter, a high-efficiency particulate air (HEPA) filter, and an activated carbon filter to remove particulate matter and O₃. The operation schedule of the indoor air purifier is shown in Figure 17. Prior to visits 1 and 3 the air purifier was absent, and prior to visits 2 and 4 the air purifier was present either with all three filters intact or only with the coarse filter (the HEPA and activated carbon filters removed). During the intervention period, the participants were suggested to close their home windows as much as possible whenever they were home. The effects of the intervention on indoor air quality have been reported previously.¹⁹⁵⁻¹⁹⁸ In the present analysis, we aim to examine relationships of airway mechanics and lung function with personal pollutant exposure in asthmatic children. The analysis is based on a panel study design as respiratory measurements were taken for each participant four times (at Visits 1-4), with two weeks in between the visits. All the visits occurred between February 14 and April 14, 2017. Efforts were made to conduct all measurements at the same time of day for all four visits.

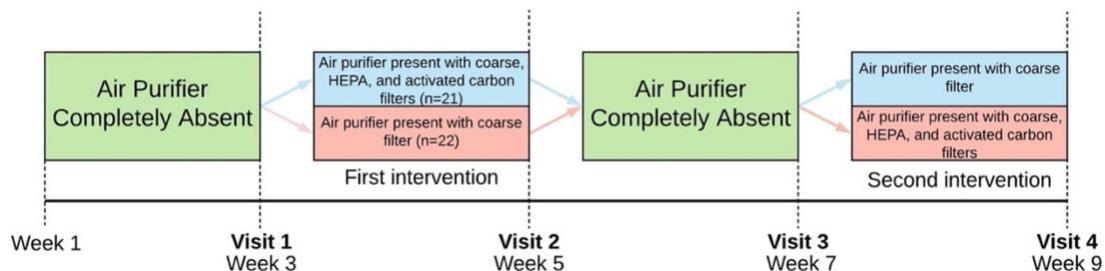


Figure 17. Air purifier operation schedule in relation to the clinic visits for physiological measurements.

5.1.2.3. Exposure assessment

PM_{2.5} and O₃ were simultaneously measured both in children’s bedrooms and outside a window of their homes. These pollutants were continuously measured using an integrated sensor box equipped with a Plantower PMS3003 sensor for PM_{2.5} and an Alphasense sensor (OX-A4) for O₃. The sensors generated the hourly averages of pollutant concentration. These sensors, validated in Beijing, Shanghai, and other cities previously,^{194, 199-201} were field calibrated in Shanghai before the start of the study and at the end of the study to account for any drift in sensor function over the duration of the study. We also obtained the ambient hourly averages of PM_{2.5}, O₃, temperature, and relative humidity during the entire study period from the government monitoring station closest to the research clinic (~9 km away), and they were summarized in Table E1. In this study, the ambient air pollutant concentrations were calculated by averaging the pollutant concentrations measured by all the outdoor sensors and the governmental monitoring station. An indoor/outdoor (I/O) ratio of 0.8 and 0.35 were used to calculate the hourly average for PM_{2.5} and O₃ concentrations,¹⁴ respectively, in other indoor

environments (e.g., classroom) based on the measurements of the ambient air pollutant concentration. In addition, pollutant concentrations in cars or subways were calculated using I/O ratios based on the literature. Different I/O ratios were applied when the participants' home windows were recorded to be open (see Table E2 in the Supplement). Combining these measured or estimated pollutant concentrations for each encountered microenvironment with detailed time-activity data (see Table E3 in the Supplement), we calculated 24-hour time-weighted personal PM_{2.5} exposures and maximum 8-hour averages of personal O₃ exposure zero to six days (lag day 0-6) prior to each clinic visit. The lag analysis is based on the consideration that air pollutants have delayed or cumulative respiratory effects on asthma morbidity.^{26, 202} We also calculated personal PM_{2.5} and O₃ exposure averaged over the two weeks prior to each clinic visit. The detailed exposure assessment is reported in the Supplement.

5.1.2.4 Health outcomes

Upon enrollment, each participant was measured for baseline weight, height, airway mechanics, and lung function. Each also received an immunoglobulin E (IgE)-mediated allergy test (Allergy Screen[®], Mediwiss Analytic GmbH Germany). Allergens including dust mite, room dust, mold, cat dander, dog dander, roach egg, milk, and shrimp were tested. The blood IgE level of 0.35 kU/L was set as the point of positive for allergic sensitization. We measured airway mechanics by impulse oscillometry (MasterScreen[™] IOS, Becton, Dickinson and Company, Germany), lung function by

spirometry (MasterScreen™ PFT system, Becton, Dickinson and Company, Germany), and fractional exhaled nitric oxide (FeNO) using a NIOX VERO with the adult mode (Circassia Pharmaceuticals Inc., USA). Indicators of airway mechanics included impedance at 5 Hz (Z_5 , increased Z_5 means higher airway impedance), resistance at 5 Hz (R_5 , increased R_5 means higher total airway resistance), resistance at 20 Hz (R_{20} , increased R_{20} means higher large airway resistance), reactance at 5 Hz (X_5 , increased X_5 negative values means worse airway resilience), and resonant frequency (F_{res} , increased F_{res} means worse airway resilience). Main spirometric lung function indicators included forced expiratory volume in the first second (FEV_1), forced vital capacity (FVC), peak expiratory flow (PEF), forced expiratory flow at 25-75% of the FVC (FEF_{25-75}), and the ratio between FEV_1 and FVC (FEV_1/FVC). Each participant attempted the impulse oscillometry and spirometry measurements until the variation among the three most recent measurements was smaller than 5%, and the highest value among these three measurements was used for data analysis.

5.1.2.5 Statistical analysis

We report means with standard deviation (SD) and medians with interquartile range (IQR) and range for participants' baseline characteristics. The Spearman correlations among personal air pollutant exposures were calculated.

The main objective of this paper is to assess the exposure-response relationships for $PM_{2.5}$ and O_3 . As personal exposure was manipulated by air filtration in the

bedrooms, we first wanted to evaluate any potential impact of “air filtration status alone”, which was to assess potential confounding from changes other than PM_{2.5} and O₃ brought by air filtration. We used linear mixed-effects regression (LMER) models in which a health outcome was the dependent variable, filtration status (no filtration versus coarse+HEPA+activated carbon filters versus coarse filters) was the independent variable; the fixed-effects covariates included 2-week average personal PM_{2.5} and O₃ exposure, 2-week average ambient temperature and relative humidity, sex, age, baseline eosinophil count, upper respiratory tract infection status, opioid cough suppressant usage, dust mite allergy status, sleep duration, asthma exacerbation status, inhaled corticosteroids usage, and travel status (whether or not traveled during the two weeks prior to each of the clinical visits). We controlled for random-effect variables including subject ID and the day of the week for clinical visit as random intercepts. From the model output, we calculated percent change (and 95% confidence interval) in the outcome following the use of the three filters together or the use of only coarse filter in reference to following the absence of any filters. The results represent the effect of filtration status alone, not the overall effect of air filtration that would integrate the effect of filtration status alone and the effect of PM_{2.5} and O₃ change resulting from the filtration.

Secondly, we used LMER models to assess the associations between personal air pollutant exposure and a health outcome. In these models, each of the health outcomes

was the dependent variable, and the personal pollutant exposure was the independent variable. We adjusted for the same covariates as described in the first model, except that the 2-week average PM_{2.5} and O₃ exposure were not included. From the model output, we calculated percent change (and 95% confidence interval) of the outcome associated with an IQR increase in personal pollutant exposure. Based on the results from the first set of models described above, we deemed it not appropriate to include the data measured following the use of only the coarse filter due to concerns on additional confounding associated with this condition. Hence, the data from three visits per person were used in the analysis of exposure-response relationships.

Thirdly, we conducted stratified analyses to assess the pollutant exposure-response relationship for pulmonary health outcomes based on low (≤ 450 / μ L) versus high (>450 / μ L) blood eosinophils number, which is a suggested cutoff point for the presence of eosinophilic asthma.²⁰³ In these models, each health outcome was the dependent variable and personal pollutant exposure was the independent variable along with the same covariate structure described in the second set of models. From each model output, we generated estimates in percent change (and 95% confidence interval) in the outcome associated with an IQR increase in pollutant exposure.

Finally, we conducted several sensitivity analyses. (1) We examined the exposure-response relationship in the data collected only in the two no-filtration visits. (2) We used co-pollutant models to examine whether the exposure-response

relationships obtained in the single-pollutant models can be retained after controlling for a co-pollutant. (3) We conducted separate analyses by excluding participants who traveled or used an inhaled corticosteroids during the two weeks prior to each of the clinical visits. (4) We conducted separate analyses by including measurement from all the clinical visits. All statistical analyses were conducted using *lme4* and *lmeTest* in R software (version 3.6.1). A P-value of 0.05 was set as the cut point for statistical significance. Based on data distributions, air pollutant exposures and some of health outcomes were natural logarithm-transformed. A detailed description of equations and codes used for the statistical models is provided in the Supplement.

5.1.3 Results

5.1.3.1 Participant characteristics

We measured 43 children with stable, mild or modest asthma (see Table 13). Among the children, 13 (30.2%) had baseline eosinophil count $> 450/\mu\text{L}$, three (7.0%) had $\text{FEV}_1/\text{Predicted FEV}_1 < 80\%$, 13 (30.2%) had FeNO larger than 20 ppb, and 35 (81.3%) were atopic and were mostly allergic to dust mite. During the study period, 9 children (20.9%) had a fever, 7 (16.3%) had asthma exacerbation, 3 (7.0%) used opioid cough suppressant, and 12 (27.8) used inhaled corticosteroids.

Table 13. Baseline characteristics of participants.

Subject Characteristics	Value
Age, mean \pm SD [range] (year)	7.8 \pm 2.3[5-13]
Female, No. (%)	17 (40%)
Weight, mean \pm SD [range] (Kg)	31.2 \pm 10.3 [19.0-59.0]
Height, mean \pm SD [range] (cm)	132.3 \pm 13.3 [110.0-166.0]
Blood eosinophil count, mean \pm SD [range] (/ μ L)	378.8 \pm 264.6 [80.0-1260.0]
FEV ₁ /FEV ₁ predicted, mean \pm SD [range] (%)	103.0 \pm 16.5 [65.5-143.0]
FVC/FVC predicted, mean \pm SD [range] (%)	104.8 \pm 12.0 [81.1-140.3]
Baseline FeNO >20 ppb, No. (%)	13 (30.2%)
Atopic, No. (%)	35 (81.3%)
Dust mite allergy, No. (%)	27 (62.7%)
Opioid cough suppressant usage, No. (%)	3 (7.0%)
Inhaled corticosteroids usage, No. (%)	12 (27.8%)

Definition of abbreviations: FEV₁/FEV₁ predicted=the ratio between FEV₁ and predicted FEV₁; FVC/FVC predicted: the ratio between FVC and predicted FVC

Table 14. Mean ± SD of personal air pollutant exposure and health outcomes for different filtration status.

	No Filtration	Filtration (Coarse + HEPA + Activated Carbon Filters)	Filtration (Coarse Filter)
PM_{2.5} (µg/m³, 24-hour average)			
Lag 0	49.0 ± 13.6	23.2 ± 11.7	36.8 ± 14.8
Lag 1	48.1 ± 16.1	21.2 ± 11.6	34.7 ± 19.3
Lag 2	43.0 ± 16.7	19.6 ± 10.4	32.5 ± 15.7
Lag 3	39.8 ± 12.2	21.1 ± 9.8	36.4 ± 16.7
Lag 4	44.0 ± 17.8	20.4 ± 9.5	35.7 ± 14.7
Lag 5	51.2 ± 23.8	20.5 ± 10.5	41.1 ± 26.8
Lag 6	50.3 ± 24.3	20.6 ± 9.2	43.9 ± 24.3
O₃ (ppb, maximum 8-hour average)			
Lag 0	22.2 ± 8.5	22.8 ± 9.0	20.2 ± 8.2
Lag 1	19.7 ± 7.6	19.3 ± 6.8	16.7 ± 6.1
Lag 2	20.8 ± 7.7	18.3 ± 4.9	18.5 ± 4.8
Lag 3	22.7 ± 7.4	18.6 ± 6.0	19.9 ± 6.4
Lag 4	22.8 ± 8.9	19.9 ± 6.5	20.4 ± 8.0
Lag 5	25.8 ± 10.4	22.0 ± 8.6	24.2 ± 10.8
Lag 6	28.0 ± 10.9	24.3 ± 10.6	26.1 ± 11.6
Airway Mechanics			
Z ₅ (cm H ₂ O/L/s)	8.8 ± 2.4	8.6 ± 2.7	9.5 ± 2.6
R ₅ (cm H ₂ O/L/s)	8.3 ± 2.3	7.8 ± 2.5	8.9 ± 2.6
R ₂₀ (cm H ₂ O/L/s)	5.6 ± 1.6	5.2 ± 1.6	5.5 ± 1.2
R ₅ -R ₂₀ (cm H ₂ O/L/s)	2.7 ± 1.7	2.7 ± 1.8	3.4 ± 2.0
X ₅ (cm H ₂ O/L/s)	-1.8 ± 2.5	-1.9 ± 2.6	-2.3 ± 2.1
Fres (Hz)	18.8 ± 6.4	18.7 ± 5.9	20.1 ± 6.5
Lung Function			
FEV ₁ (L)	1.7 ± 0.5	1.7 ± 0.5	1.7 ± 0.5
FVC (L)	2.1 ± 0.6	2.1 ± 0.6	2.1 ± 0.7
PEF (L/s)	3.8 ± 1.2	3.8 ± 1.1	3.9 ± 1.1
FEF ₂₅₋₇₅ (L/s)	1.7 ± 0.6	1.7 ± 0.6	1.7 ± 0.6
FEV ₁ /FVC	83.1 ± 8.6	83.1 ± 7.3	83.1 ± 8.1
Airway Inflammation			
FeNO (ppb)	22.7 ± 20.4	21.9 ± 18.9	25.7 ± 19.3

Definition of abbreviations: Z₅=airway impedance measured at 5Hz; R₅=airway resistance measured at 5Hz; R₂₀=airway resistance measured at 20Hz; R₅-R₂₀=difference

between airway resistance measured at 5Hz and 20Hz; X_5 =airway reactance measured at 5Hz; F_{res} =resonant frequency; FEV_1 =forced expiratory volume in first second; FEV_1/FEV_1 predicted=the ratio between FEV_1 and predicted FEV_1 ; FVC=forced vital capacity; FVC/FVC predicted: the ratio between FVC and predicted FVC; PEF: peak expiratory flow; FEF_{25-75} =the average forced expiratory flow during 25% to 75% of FVC; FEV_1/FVC =the ratio between FEV_1 and FVC; FeNO=fractional exhaled nitric oxide.

5.1.3.2 Air pollutant exposure in relation to filtration status

Participants spent 12.3 ± 2.8 hours per day in their bedrooms, which provided an opportunity to generate a wide range of personal pollutant exposures by manipulating the bedroom air pollutant concentration through air filtration. In Table 14, 24-hour average personal $PM_{2.5}$ and O_3 exposure 0-6 days prior to each clinic visit are compared. When the clinic visits occurred following no filtration, the mean personal $PM_{2.5}$ exposures were the highest. When the clinic visits occurred following the use of the three filters, the mean $PM_{2.5}$ exposures were the lowest. When the clinic visits occurred following the use of the coarse filter only, the mean exposures were in the middle. The maximum daily 8-hour average personal O_3 exposure 0-6 days prior to a clinic visit did not differ substantially by filtration status. The results indicate that by operating bedroom air purification, a wider range in personal $PM_{2.5}$ exposure was obtained, while not for O_3 exposure. As shown in Figure E1, there were no strong correlations between $PM_{2.5}$ exposure and O_3 exposure.

5.1.3.3 Health outcomes in relation to filtration status

As shown in Table 14, the participants' airway mechanics were the worst after the 2-week use of only the coarse filter even though concentrations of $PM_{2.5}$ were lower

during this period than they were in the absence of any filtration. The effects of “filtration status alone”, independent of the effect of PM_{2.5} and O₃ changes resulting from the filtration, are shown in Figure 18. We found that using only the coarse filter was associated with increases in Z₅, R₅, and R₅-R₂₀ by 8.4% (95%CI: -0.2%-16.9%), 9.7% (-0.9% - 20.3%), and 27.6% (0.5%-54.7%), respectively, compared with using no filtration at all. However, none of the outcomes changed significantly when comparing the visits following the use of all the three filters to the visits following the absence of any filtration. The filtration status did not show a significant effect on the spirometric lung function measures and FeNO, although it appears that the use of coarse filter was associated with a 12.5% (-4.9%-33.0%) increase in FeNO (Figure 18). Please note that these “filtration status alone” effects, excluding the effect of PM_{2.5} reduction caused by the filtration, do not reflect the overall (net) effect of filtration.

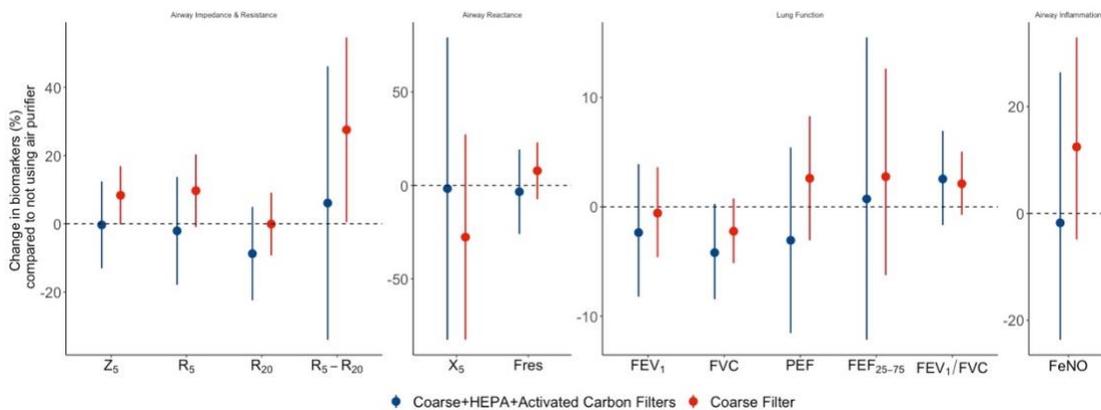


Figure 18. Change in biomarkers (%) measured from after using no air purifier to using either air purifier with all the three filters or with only the coarse filter.

5.1.3.4 Exposure-response relationship

Concerning the effects of potential co-pollutants exposure as shown above, we excluded the visits following the use of coarse filter in the exposure-response analysis. Using the data from the other three visits, as shown in Figure 19, we found that an IQR ($30.3 \mu\text{g}/\text{m}^3$) increase in 24-hour personal $\text{PM}_{2.5}$ exposure one day prior to the clinic visits was significantly associated with increases in total airway resistance (R_5) of 6.3% (0.1%-12.5%), small airway resistance (R_5 - R_{20}) of 15.8% (95%CI: 0.4%-31.1%), and fractional exhaled nitric oxide (FeNO) of 9.6% (0.7%-19.3%). In addition, IQR increases in 24-hour $\text{PM}_{2.5}$ exposure measured zero days ($27.1 \mu\text{g}/\text{m}^3$), three days ($22.8 \mu\text{g}/\text{m}^3$), four days ($29.2 \mu\text{g}/\text{m}^3$), and five days ($38.9 \mu\text{g}/\text{m}^3$) prior to the clinic visits were associated with increases in FeNO of 12.1% (1.3% to 24.2%), 20.6% (8.9% to 33.5%), 18.2% (8.4% to 28.9%), and 25.3% (9.5% to 43.4%), respectively. We did not observe a significant association between any prior-day $\text{PM}_{2.5}$ exposure and any of the lung function measures. We did not find a significant or a clear trend in association between O_3 exposure and any of the health outcomes (Figure 19).

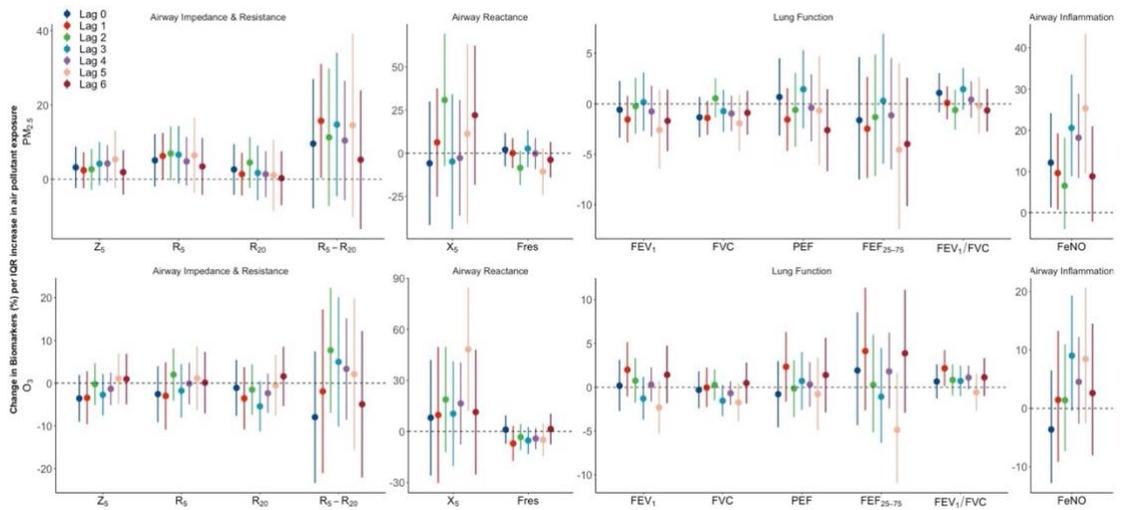


Figure 19. Change in biomarkers (%) with one IQR increase in 24-hour average PM_{2.5} and O₃ personal exposure in zero to six days prior to health outcome measurement.

5.1.3.5 Effect modification

We considered baseline blood eosinophil count as a potential effect modifier. In stratified analyses, we found that the increase in personal PM_{2.5} exposure was associated with significant increases in Z₅, R₅, R₅-R₂₀, and FeNO only in children with lower blood eosinophil counts ($\leq 450/\mu\text{L}$) (Figure 20). In children with higher blood eosinophil counts, the exposure-response associations were non-significant and unclear, except that an increase in PM_{2.5} exposure five days prior to the clinic visits was associated with significant decreases in Z₅ and R₅, and a significant increase in FEV₁.

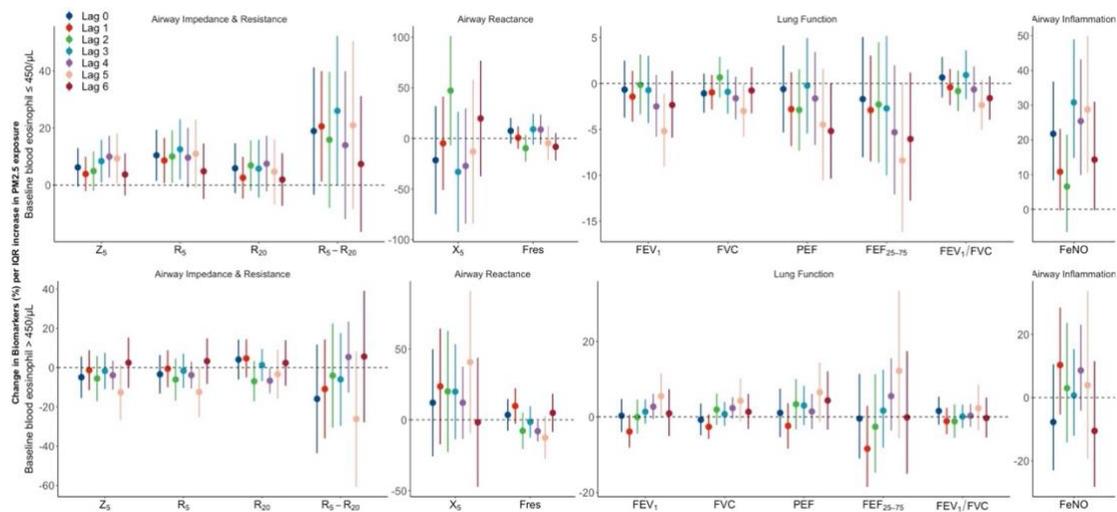


Figure 20. Change in biomarkers (%) with one IQR increase in 24-hour average PM_{2.5} personal exposure in zero to six days prior to health outcome measurement.

5.1.4. Discussion

The principal finding of this study is that day-to-day increases in personal PM_{2.5} exposure were associated with worsening airflow limitation in asthmatic children due to increased airway resistance and pulmonary inflammation. As improving airflow is a key challenge in asthma management, these associations point to the importance of reducing personal PM_{2.5} as a practical means of asthma control especially for those living in places with higher concentrations of ambient and/or indoor PM_{2.5}.

In the present study, we found that increased PM_{2.5} exposures measured several days prior were associated with significantly increased FeNO, confirming that FeNO is a sensitive biomarker of air pollutant induced airway inflammation.²⁰⁴ However, the associations of spirometric lung function and PM_{2.5} exposure were non-significant and unclear (see Figure 19). The results were supported by previous studies. For example, one study reported that an IQR (24.0 μg/m³) increase in personal PM_{2.5} exposure

averaged over 48-hours prior to the health outcome measurement (mean \pm SD: $36.2 \pm 25.5 \mu\text{g}/\text{m}^3$) was associated with a significant increase in FeNO of 4.2% in asthmatic children.³³ Another study reported that increased PM_{2.5} exposure 24-hour prior to the clinical visit (mean \pm SD: $31.2 \pm 21.8 \mu\text{g}/\text{m}^3$) was not associated with a change in FEV₁ in asthmatic children.²⁷ In contrast, Delfino et al., 2004 found that an IQR ($30.3 \mu\text{g}/\text{m}^3$) increase in personal PM_{2.5} exposure during the preceding 24-hour (mean \pm SD: $37.9 \pm 19.9 \mu\text{g}/\text{m}^3$) was associated with a decrease in FEV₁ of 5.9% in children with asthma.²⁶ Although the PM_{2.5} exposure concentrations reported in Delfino et al., 2004 are similar to those of the current study (lag 0 mean \pm SD: $39.5 \pm 17.1 \mu\text{g}/\text{m}^3$), other factors including differences in demographic characteristics, study design, changes or ranges in exposure concentrations in different association analyses, and potential confounders might contribute to the inconsistency.

To the best of our knowledge, this is the first study to examine the relationships of personal O₃ exposure, when ambient ozone concentrations were relatively low during a non-ozone season (daily maximum 8-hour ambient O₃ concentration range: 12.6 - 93.4 ppb), with lung function and FeNO in asthmatic children. As expected at these low concentrations, ozone exposure was not clearly or significantly associated with lung function and FeNO in the present study. In contrast, at relatively higher exposure levels, with daily maximum 8-hour ambient O₃ concentration ranging from 63.3 - 99.6 ppb, a previous study conducted during an ozone season of Southern New England reported

that an increase in maximum 8-hour ambient ozone concentration measured in 24-hour and one day prior to the clinical visit were associated with worsening lung function in asthmatic children.²⁰⁵

The non-significant associations of spirometric lung function with PM_{2.5} and O₃ exposure in the present study suggest that the concentration and/or duration of pollution exposure in this study might not be adequate to induce functional changes in an asthmatic lung. However, we found that, as the indicators of the early-stage and subtle changes in lung function,²⁸ total (R₅) and small airway resistance (R₅-R₂₀) were associated with personal PM_{2.5} exposure measured several days prior to the outcome measurements. The adverse effects of air pollutant exposure on airway mechanics of healthy children and adults have been previously reported. For example, one study compared the airway mechanics of children from two cities in India, finding that children who lived in the city with higher ambient air pollutant concentrations had worse airway resistance (R₅) and airway reactance (X₅).²⁰⁶ Another study reported that overnight filtration of bedroom air was associated with significant decreases in airway resistance (R₅) and impedance (Z₅) in healthy adults.⁷¹ In addition to the previous findings, the present study provides the first evidence to support a significant exposure-response relationship of personal PM_{2.5} exposure with total and small airway resistance.

As total airway resistance is the sum of small and large airway resistance, the results of this study led us to hypothesize that the increases in PM_{2.5}-induced total

airway resistance might be largely driven by the increased small airway resistance. The small airways, anatomically defined as airways with inner diameters less than two millimeters, cumulate greater cross-sectional area than the large airways.²⁰⁷ The small airways are the major sites of airflow obstruction and contribute substantially to airway inflammation in asthma.^{208, 209} PM_{2.5} in inhaled air can penetrate and be retained in the walls of the small airways,²¹⁰ resulting in higher oxidative stress levels and inflammation in the respiratory tract.²¹¹ Therefore, decreasing personal exposure to PM_{2.5}, which would lead to less PM_{2.5} entering the small airways, would be important in asthma management.²¹²

Air purifiers with HEPA and activated carbon filters have been reported to be efficient at removing PM_{2.5} and O₃, respectively.^{213, 214} In the present study, personal exposures to PM_{2.5} were noticeably manipulated by the operation of an indoor air purifier, although O₃ did not vary substantially by filtration status, potentially due to the low ambient O₃ concentration during the study period (see Table 14). On the other hand, we found that, independent of the effect of PM_{2.5} and O₃ changes resulting from the filtration, using an air purifier with only the coarse filter was associated with worsened airway mechanics compared to using no filtration. This might reflect confounding from indoor-generated pollutants such as volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs), some of which (e.g., formaldehyde) are known respiratory toxicants.²¹⁵ During each air filtration period, the participants were asked to

close their home windows as much as possible, while it is not required during the no-filtration periods. Indoor VOC and SVOC concentration were expected to be higher when the windows were closed more often, as window closure would reduce indoor-outdoor air exchange and consequently enhanced the accumulation of indoor-generated pollutants.²¹⁶ However, VOCs and SVOCs can be captured, at least partly, by the activated carbon filter and the HEPA filter. Hence, we speculate that the window closure influence on indoor levels of VOCs and SVOCs was smaller for the three-filter condition than for the coarse-filter only condition.

Asthma is a phenotypically heterogeneous disease which can be characterized by eosinophilic, neutrophilic, or mixed eosinophilic/neutrophilic inflammatory patterns ²¹⁷. Previous studies have reported that patients with eosinophilic asthma are more responsive to allergic sensitization,²¹⁸ while non-eosinophilic (or neutrophilic) asthma is mainly triggered by environmental exposures including bacterial endotoxin and air pollutants.²¹⁹ In line with previous findings, in this study, we observed that day-to-day increases in personal PM_{2.5} exposure were associated with significant increases in airway impedance, resistance, reactance, and inflammation mainly in children with non-eosinophilic asthma (i.e., those with baseline blood eosinophils number less than 450/ μ L). These results suggest that phenotype-targeted therapies based on eosinophilic or non-eosinophilic inflammatory patterns would be important for asthma management.

5.1.4.1 Limitations and sensitivity analyses

Our assessment of the coarse-filter only data suggests a potential role of co-pollutant exposures, such as VOCs and SVOCs, in affecting airway mechanics and FeNO. However, we were not able to measure these co-pollutants in the bedrooms. Our findings on PM_{2.5} effect may be subject to confounding of co-pollutants other than ozone that was measured and accounted for. To address this issue, we assumed that the combined use of the activated carbon filter and the HEPA filter would have offset increases in indoor VOCs and SVOCs resulting from increased window closure during filtration period. However, if concentrations of these co-pollutants were different between the three-filter filtration period and the no-filtration period, this could have confounded the PM_{2.5} exposure-response relationship. With this in mind, we analyzed the data collected only in the two no-filtration visits and found that increased PM_{2.5} exposure measured during the 24-hours prior to the clinical visit was associated with significant decreases in FEV₁ and FVC and statistically non-significant increases in airway impedance (Z_5) and inflammation (FeNO) (Figure E2). This analysis, albeit with reduced statistical power, supports the robustness of the main findings from the three-visits analysis.

Another limitation of this study is that the personal air pollutant exposure was calculated by coupling pollutant concentrations in microenvironments and participants' time activity patterns. Although this calculation might introduce systematic or random

error into the statistical analyses of this study, this is a more logistically feasible exposure assessment method than personal monitoring in this study design and for these study participants.

We further evaluated the robustness of the results through two sensitivity analyses. Firstly, there were not remarkable changes in the relationships shown in the co-pollutant models in terms of either statistical significance or effect size (Figure E3-E4). Similarly, after excluding measurement of participants who traveled or used inhaled corticosteroids during the two weeks prior to each of the clinical visits, the analysis showed similar results (Figure E5-E6), supporting the robustness of the findings from the main analyses. The PM_{2.5} exposure-response relationships were examined including all the clinical visits. As shown in Figure E7, we found that day-to-day changes in personal PM_{2.5} exposure was associated with decreased lung function and increased airway resistance and airway inflammation, supporting the findings of the main analyses.

5.1.5 Conclusion

Although insufficient to be significantly associated with changes in lung function, day-to-day changes in personal exposure to PM_{2.5}, resulting partly from the use of a bedroom air purifier, were significantly and adversely associated with changes in small airway and total airway resistance as well as pulmonary inflammation in children with asthma. These associations were not affected by the co-presence of ozone at low

levels when ozone itself was not associated with any of the measured outcomes. Our findings suggest the importance of reducing personal exposure to PM_{2.5} as part of the asthma management plan to improve airflow limitation.

5.2 Part B: Malondialdehyde in Nasal Fluid: A Biomarker for Monitoring Asthma Control in Relation to Air Pollution Exposure.

This chapter is adapted with permission from He, L.; Cui, X.; Li, Z.; Teng, Y.; Barkjohn, K. K.; Norris, C.; Fang, L.; Lin, L.; Wang, Q.; Zhou, X.; Hong, J.; Li, F.; Zhang, Y.; Schauer, J. J.; Black, M.; Bergin, M.; Zhang, J., Malondialdehyde in Nasal Fluid: A Biomarker for Monitoring Asthma Control in Relation to Air Pollution Exposure. *Environmental Science and Technology*. 2020 (Publisher: American Chemical Society). The accompanying supporting information is included in Appendix F. In this study and the published manuscript, I conducted the air pollution exposure assessment, analyzed nasal MDA, conducted the statistical analysis, interpretate the results, design all tables and figures, and led the writing and revising of the manuscript. The coauthors contributed to the study design (JZ, ZL, JS, MB, and MHB), funding acquisition (JZ, MHB, JS, ZL, and YZ), manuscript revisions (JZ), sample and data collection (ZL, YT, XC, KB, CN, LF, LL, QW, XZ, JH, FL, YZ, JS, MHB, and JZ), sample analysis (XC and YT), and discussions (JZ).

5.2.1 Introduction

Air pollutants, including particulate matter with an aerodynamic diameter ≤ 2.5 μm (PM_{2.5}) and ozone (O₃), are well-established risk factors for asthma exacerbation.²² Elevated PM_{2.5} and O₃ levels are found in polluted urban atmospheres worldwide, and the infiltration of outdoor pollutants may further elevate the indoor pollutant levels.¹⁸⁹

²²⁰ Exposure to these pollutants increases oxidative stress in the human body, which is a pathophysiologic pathway for asthma exacerbation.²²¹

Previous epidemiologic studies have reported associations of air pollution exposure with oxidative stress in the circulatory system and the lower respiratory tract in patients with asthma.²²²⁻²²⁴ Increased oxidative stress in the lower respiratory tract has also been associated with increased airway inflammation, a hallmark of asthma exacerbation.²²⁵ We hypothesize that O₃ and/or PM_{2.5} can exert oxidative damage in the nose upon entering the respiratory tract. This damage may trigger a mucosal irritating sensation, exacerbating the symptoms of asthmatic patients. However, no studies have explored an oxidative stress biomarker in nasal fluid in relation to air pollution exposure.

Malondialdehyde (MDA), a stable product of reactive oxygen species (ROS)-induced lipid peroxidation, has been widely used as a biomarker of oxidative stress. It has been measured in urine and exhaled breath condensate to indicate oxidative stress in the circulatory system and lower respiratory tract, respectively.^{112, 226} However, there have been no studies that examined potential associations of air pollutant exposure with MDA in nasal fluid. Nasal MDA may serve as a biomarker of oxidative stress in upper respiratory tract, given that the nose is a prime portal of entry of air pollutants into the human body.³⁴ Hence, we hypothesize that increasing personal exposure to PM_{2.5} and/or O₃ would increase MDA concentrations in nasal fluid and that nasal MDA would be

associated with asthma symptom scores collected via the Childhood Asthma-Control Test (C-ACT).^{227, 228}

To test these hypotheses, we used data collected from a cohort of asthmatic children and conducted statistical analyses to examine the relationships between (1) pollutant exposures and nasal MDA, (2) pollutant exposures and C-ACT scores, and (3) nasal MDA and C-ACT scores. In addition, as ROS-induced oxidative stress in the upper respiratory tract may spill over to the circulatory system and lower respiratory tract, we measured two biomarkers of systemic oxidative stress (urinary MDA and 8-hydroxy-2'-deoxyguanosine [8-OHdG]) and a biomarker of pulmonary inflammation (fractional exhaled nitric oxide [FeNO]) and evaluated their associations with pollutant exposure and C-ACT scores. These additional analyses provided insight about which of the nasal and systemic biomarkers predict asthma control symptom scores in our study cohort.

5.2.2 Methods

5.2.2.1 Study participants and baseline measurements

We recruited 43 children (17 girls and 26 boys), 5 to 13 years old, from a pool of asthmatic children at Shanghai General Hospital, located in Songjiang, a suburb of Shanghai, China. Children were eligible to participate if they had been diagnosed with mild or moderate asthma and had at least one asthma attack during the past 12-month. Children with chronic diseases other than asthma were not eligible. Upon enrollment, 13

children had eosinophilic inflammation, indicated by having baseline eosinophil count > 450/ μ L, and 35 were atopic and were mostly allergic to dust mite.

Oral assent was obtained from each child participant and written consent was obtained from the child's caregiver (a parent in majority of cases). The study protocol was approved by the Ethics Committee of Shanghai General Hospital and Duke University Campus IRB.

5.2.2.2 Study design

The detailed study design has been published previously.⁷² Briefly, this study used data collected in an indoor air filtration intervention, where indoor PM_{2.5} and O₃ concentrations were manipulated through the operation of a portable air purifier (Atmosphere®, Amway, USA) in children's bedrooms for two weeks at a time for two times. The two intervention periods were separated by a two-week washout period. The air purifier was equipped with a coarse filter, a high-efficiency particulate air (HEPA) filter, and an activated carbon filter. The effects of the intervention on indoor air quality have been previously reported.^{193-198, 229} The operation schedule of the air purifier is shown in Figure F1. In the present study, the analysis is based on a panel study design in which children were clinically assessed four times from February 14 to April 14, 2017. Specifically, there were two visits for each two-week intervention period, with one at the beginning and one at the end of intervention. Whenever possible, the 4 clinical visits took place almost at the same time of the day.

5.2.2.3 Air pollution exposure assessment

We simultaneously measured PM_{2.5} and O₃ in children's bedrooms and outside a window of their homes. These pollutants were continuously measured using a sensor box equipped with an Alphasense sensor (OX-A4) for O₃ and a Plantower PMS3003 sensor for PM_{2.5}, and hourly averages of pollutant concentrations were generated. The sensors have been previously validated in various cities and field calibrated before and after the study period.^{194, 199-201} Hourly averages of ambient temperature, relative humidity, PM_{2.5}, and O₃ concentrations were obtained from the governmental environmental monitoring station closest to the research clinic (~9 km away) where children were assessed by a physician. In the present study, to achieve a more precise estimation of ambient air pollutant concentrations, we further calculated them by averaging the concentrations measured by the monitoring station and our outdoor sensors. We used various indoor/outdoor (I/O) ratios (see Table F1), according to the literature data for similar building structures, to calculate pollutant concentrations in different indoor microenvironments where no actual pollutant measurements were obtained.^{71, 175} Coupling the microenvironmental concentrations and detailed time-activity data, we calculated 24-hour average personal PM_{2.5} exposure and daily maximum 8-hour average personal O₃ exposure zero to six days (lag day 0-6) prior to biospecimen collection. This lag analysis was used in considering that air pollutants

have cumulative or delayed respiratory effects on asthma morbidity.²⁶ The detailed exposure assessment can be found in a previous publication.¹⁵⁰

5.2.2.4 Biospecimens relevant to the present study

During each clinic visit, children provided nasal fluid and urine samples. The nasal fluid was collected using a mixed cellulose ester sampling strip (HAWG 047S6, Millipore Sigma, USA) (5mm wide and 40 mm long).²³⁰ During sampling, a strip was inserted into the nostril and taken out after two minutes. Both nostrils were sampled simultaneously. After sampling, the strips were immersed in 300 μ L deionized water and vortexed for two minutes. They were then centrifuged for two minutes at 6,600 rpm, and the supernatant was aliquoted and stored at -20 °C prior to analysis, which was done within two months of collecting the samples.²³¹ Urine samples were stored at -80 °C prior to analysis for biomarkers.

We analyzed MDA in nasal fluid as a potential biomarker of oxidative stress in the upper respiratory tract using a method described previously.¹¹² Briefly, MDA was derivatized with thiobarbituric acid (TBA) to generate the MDA-TBA adduct. The adduct was then analyzed by high-pressure liquid chromatography (HPLC) coupled with fluorescence detection. We used averages of MDA concentrations from both nostrils for data analysis. FeNO, as the biomarker of airway inflammation, was measured using a NIOX VERO (Circassia Pharmaceuticals Inc., USA). We also measured two biomarkers of systemic oxidative stress. The first is urinary MDA, which was

measured using the same method described above. The second is urinary 8-OHdG, a stable product of free radical induced oxidative DNA lesion.^{232, 233} We also measured urinary 6-sulfatoxymelatonin (aMT6s), a surrogate of circulating melatonin, to adjust for the potential effects of natural diurnal variation in melatonin levels on the relationships of air pollutant exposure with biomarkers of systemic oxidative stress.^{154, 234} Urinary 8-OHdG and aMT6s were simultaneously measured using a HPLC-tandem mass spectrometry method previously reported.¹²¹

5.2.2.5 Childhood Asthma-Control Test (C-ACT)

The C-ACT used in this study was developed by GlaxoSmithKline (GSK) and validated in children 4 to 11 years old.²³⁵ In this study, thirty-seven (86%) children, 5 to 11 years old, filled out the C-ACT with their caregivers four times – once during each of the clinical visits. As shown in Table 15, the test included four questions answered by children on asthma control, limitation of physical activities, coughing, and waking up at night recently (each with scores ranging from 0-3). The C-ACT also included three questions for caregivers on their children's daytime symptoms, daytime wheezing, and waking up at night during the past four weeks (each with scores ranging from 0-5).²³⁶ A higher individual symptom score means improved asthma symptoms, and a higher total C-ACT score indicates a better overall control of asthma.

Table 15. The questions in the C-ACT questionnaire.

Answered by child with asthma (scores: 0: Very bad, 1: Bad, 2: Good, 3: Very good)	
1	[Asthma control] How is your asthma today?
2	[Limitation of physical activities] How much of a problem is your asthma when you run, exercise or play sports?
3	[Coughing] Do you cough because of your asthma?
4	[Waking up at night] Do you wake up during the night because of your asthma?
Answered by caregiver. (scores: 0: Everyday, 1: 19-24 days/month, 2: 11-18 days/month, 3: 4-10 days/month, 4: 1-3 days/month, 5: Not at all)	
5	[Daytime asthma symptoms] During the last 4 weeks, on average, how many days per month did your child have any daytime asthma symptoms?
6	[Wheezing] During the last 4 weeks, how many days per month did your child wheeze during the day because of asthma?
7	[Waking up at night] During the last 4 weeks, on average, how many days per month did your child wake up during the night because of asthma?

5.2.2.6 Statistical analyses

Concentrations for all the biomarkers and for air pollutants had a skewed distribution. Hence, we used natural logarithm-transformed data in statistical models described below.

First, we used Linear mixed-effect regression (LMER) models to investigate the associations of personal pollutant exposure with biomarkers (nasal MDA, FeNO, urinary MDA & 8-OHdG) and with C-ACT scores. In these models, biomarker or C-ACT score was the dependent variable, and the personal air pollutant exposure was the independent variable. We controlled for the fixed-effects covariates including ambient

temperature, ambient relative humidity, urinary aMT6s (only for the 2 urinary biomarkers), respiratory tract infection status, inhaled corticosteroids usage, physician-diagnosed asthma exacerbation status, baseline eosinophil count, sleep duration, travel status (whether or not the participant was out of city during the two weeks prior to each clinical visit), sex, and age. We controlled for subject ID and the day of the week for clinical visit as random-effect variables. From the model output, we calculated percent change (and 95% confidence interval) in biomarker or C-ACT score associated with an IQR increase in personal pollutant exposure.

Second, we used LMER models to examine the associations of C-ACT score with biomarker concentrations. In these models, the total score or individual question score was the dependent variable, and the concentration of each biomarker was the independent variable, along with the same covariate structure described in the above models. From the model output, we calculated percent change (95% confidence interval) in C-ACT score associated with an IQR increase in biomarker concentration.

Finally, we conducted several sensitivity analyses. (1) We used co-pollutant models to examine whether the results obtained in the single-pollutant models can be retained after controlling for the co-pollutant. (2) We conducted separate analyses on participants who had asthma exacerbation or used inhaled corticosteroids during the two weeks prior to the clinical visit. (3) We tested whether asthma exacerbation status, inhaled corticosteroids usage, eosinophilic inflammation status, or sex would modify the

associations of nasal MDA with pollutant exposure. The statistical analyses were conducted using *lme4* and *lmeTest* in R software (version 3.6.1). We used a P-value of 0.05 as the cut point for statistical significance. Detailed model results (i.e. effect size, 95% confidence interval, and p-value) are shown in Tables F2-F4. Detailed descriptions of codes and equations used for the statistical analyses are provided in the Supplement.

5.2.3 Results

5.2.3.1 Subject characteristics

The detailed subject characteristics have been reported previously and shown in Table 16.^{72, 150} Among the 172 measurements (43 children * 4 visits/child), 31 (18%) were accompanied by the use of inhaled corticosteroid, 7 (4%) by asthma exacerbation, and 64 (37%) by respiratory tract infection-like symptoms during the two weeks prior to clinical visits.

Table 16. Baseline characteristics of participants.

Subject Characteristics	Value
Age, mean \pm SD [range] (year)	7.8 \pm 2.3[5-13]
Female, No. (%)	17 (40%)
Height, mean \pm SD [range] (cm)	132.3 \pm 13.3 [110.0-166.0]
Weight, mean \pm SD [range] (Kg)	31.2 \pm 10.3 [19.0-59.0]
Blood eosinophil count, mean \pm SD [range] (/ μ L)	378.8 \pm 264.6 (80.0-1260.0)

Table 17. Statistical summaries of personal air pollutant exposure.

	Mean ± SD	Median [IQR]	Range
PM_{2.5} (µg/m³, 24-hour average)			
Lag 0	39.5 ± 17.1	38.1 [24.8]	9.0 – 86.1
Lag 1	38.0 ± 19.5	37.7 [27.2]	4.9 – 111.5
Lag 2	34.5 ± 18.0	30.1 [31.0]	3.9 – 76.6
Lag 3	34.3 ± 14.5	32.4 [22.2]	6.2 – 82.2
Lag 4	36.1 ± 18.1	33.1 [27.2]	0.5 – 92.2
Lag 5	40.9 ± 25.4	37.2 [33.5]	3.7 – 181.3
Lag 6	40.9 ± 24.8	37.0 [30.7]	1.9 – 139.0
O₃ (ppb, maximum 8-hour average)			
Lag 0	21.8 ± 8.5	20.8 [9.3]	6.1 – 52.9
Lag 1	18.9 ± 7.1	18.4 [8.5]	5.4 – 46.1
Lag 2	19.6 ± 6.5	19.1 [7.7]	5.6 – 51.5
Lag 3	20.9 ± 7.0	20.2 [8.2]	6.3 – 51.1
Lag 4	21.4 ± 8.2	19.6 [8.1]	1.1 – 56.4
Lag 5	24.4 ± 10.2	22.1 [13.4]	8.5 – 52.3
Lag 6	26.6 ± 11.0	24.4 [14.5]	4.5 – 56.5

Table 18. Statistical summaries of biomarker concentration and C-ACT scores.

	Mean ± SD	Median	Range
Oxidative Stress in upper respiratory tract			
Nasal MDA (ng/mL)	7.1 ± 7.0	4.7 [7.6]	0.6 – 43.4
Airway inflammation			
FeNO (ppb)	23.6 ± 20.0	17.0 [21]	5.0 – 120
Systemic oxidative stress			
Urinary MDA (ng/mg creatinine)	624 ± 691	422 [393]	71.5 – 6367
Urinary 8-OHdG (ng/mg)	12.5 ± 49.9	1.9 [7.4]	0.01 – 592
C-ACT: reported by child			
Asthma control	2.6 ± 0.5	3.0 [1.0]	1.0 – 3.0
Limitation of physical activities	2.5 ± 0.7	3.0 [1.0]	0 – 3.0
Coughing	2.3 ± 0.8	2.0 [1.0]	0 – 3.0
Waking up at night	2.8 ± 0.4	3.0 [0]	2.0 – 3.0
Total	10.2 ± 1.8	10.0 [3.0]	4.0 – 12.0
C-ACT: reported by caregiver			
Daytime asthma symptoms	4.7 ± 0.7	5.0 [1.0]	0 – 5.0
Wheezing	4.8 ± 0.5	5.0 [0]	2.0 – 5.0
Waking up at night	4.9 ± 0.4	5.0 [0]	3.0 – 5.0
Total	14.4 ± 1.3	15.0 [1.0]	8.0 – 15.0

5.2.3.2 Exposure-response relationship

The day-to-day changes in air pollutant exposures are shown in Table 17, and there were no strong correlations between PM_{2.5} and O₃ exposure (Figure F2). Using the air pollutant exposures and biomarker concentrations shown in Table 18, we examined the relationships of air pollutant exposures with nasal MDA, FeNO, and urinary MDA &

8-OHdG. As shown in Figure 21, we found that an IQR increase in 24-hour personal PM_{2.5} exposure measured 0, 1, 2, 3, 4, and 5 days prior to the clinic visits were associated with increased concentration of MDA in the nasal fluid by 38.6% (95% CI: 14.3% to 68.1%), 39.2% (16.4% to 66.6%), 51.0% (19.1% to 91.5%), 54.9% (20.6% to 99.1%), 49.8% (20.3% to 86.5%), and 42.3% (8.1% to 87.5%) respectively. However, the associations of PM_{2.5} exposure with urinary MDA and 8-OHdG were non-significant in this study. In addition, an IQR increase in 24-hour PM_{2.5} exposure measured four days prior was associated with the increase in FeNO of 10.2% (1.2% to 20.0%). Besides, IQR increases in maximum daily 8-hour average O₃ exposure measured two days, three days, and four days prior were associated with significantly increased nasal MDA by 22.1% (0.9% to 47.9%), 69.4% (40.3% to 104.6%), and 25.1% (4.6% to 49.7%), respectively. We did not find a clear trend in the associations between O₃ exposure and any of the two systemic oxidative stress biomarkers and FeNO.

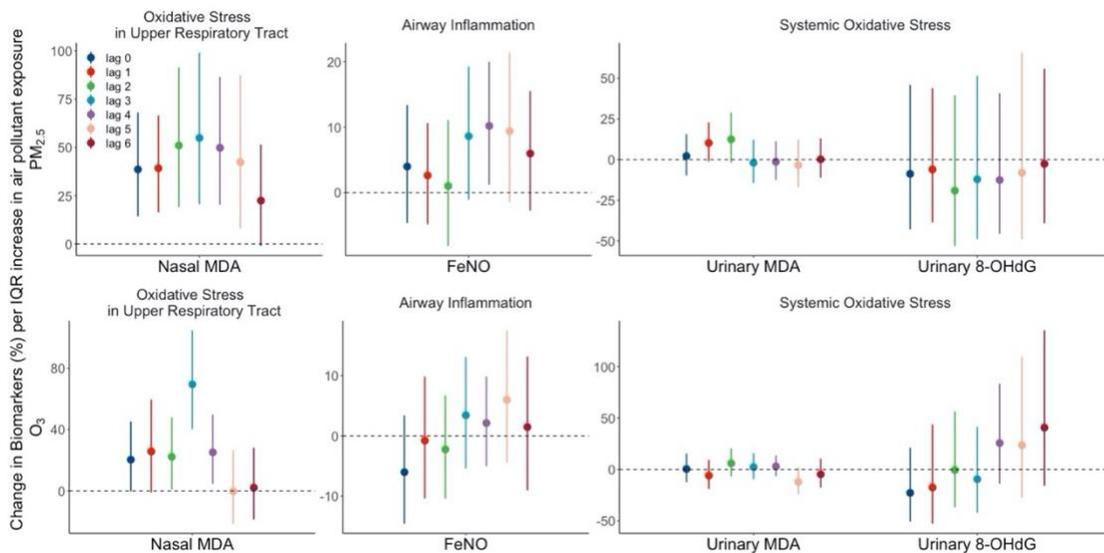


Figure 21. Change in biomarkers (%) with one IQR increase in 24-hour average PM_{2.5} and maximum daily 8-hour average O₃ personal exposure.

The associations of pollutant exposure with C-ACT scores are shown in Figure 22. We found that day-to-day increases in personal PM_{2.5} and those in O₃ exposure were associated with decreased individual symptom score and total C-ACT scores, respectively. Specifically, IQR increases in 24-hour personal PM_{2.5} exposure measured one day and four days prior to the clinic visits were associated with significantly decreased total C-ACT score (reported by child) by 4.7% (-8.2% to -1.2%) and 4.6% (-8.6% to -0.7%) respectively. Similarly, an IQR increase in PM_{2.5} exposure measured five days prior was associated with significantly decreased total C-ACT score (reported by caregiver) by 3.7% (-6.0% to -1.3%). In addition, we found that PM_{2.5} exposure measured one day, two days, four days, and five days prior were associated with significantly decreased individual score for asthma control. Increasing PM_{2.5} exposures measured one

day, four days, or five days prior were significantly associated with decreased individual scores for limitation of physical activities, coughing, waking up at night (reported by child), daytime asthma symptoms, and wheezing, respectively. Besides, we found that the increase in maximum daily 8-hour average O₃ exposure measured one day, two days, three days, four days, five days, and six days prior was associated with a significantly decreased score for waking up at night (reported by the child). In addition, increasing O₃ exposure measured one day prior was associated with a decreased score for daytime asthma symptoms. However, the relationships between O₃ exposure and other C-ACT outcomes were inconclusive and non-significant.

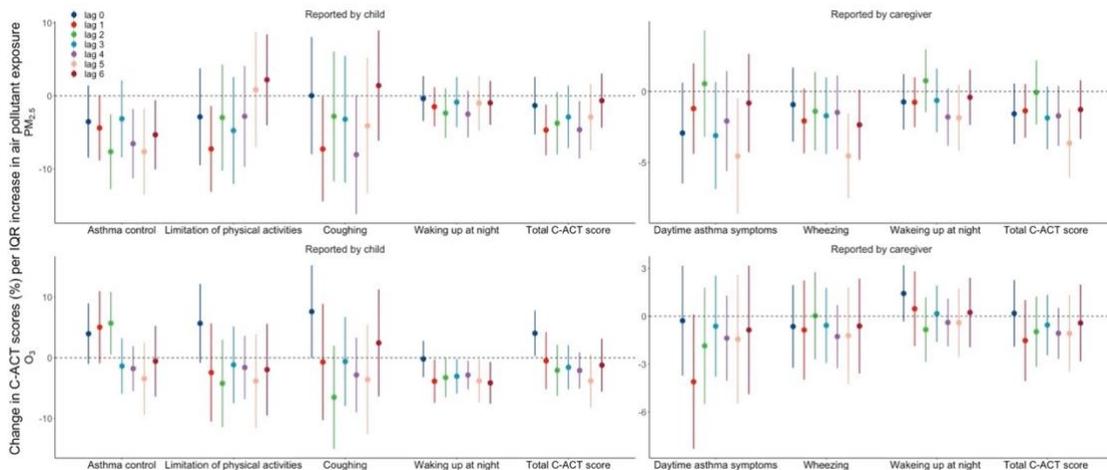


Figure 22. Change in C-ACT outcomes (%) with one IQR increase in 24-hour average PM_{2.5} and maximum daily 8-hour average O₃ personal.

5.2.3.3 Relationship between C-ACT scores and biomarker concentrations

The associations of C-ACT scores with nasal MDA, FeNO, and urinary MDA & 8-OHdG are shown in Figure 23. We found that an IQR increase in nasal MDA was associated with significantly decreased scores for limitation of physical activities,

coughing, and total C-ACT score (reported by child) by 8.8% (-16.3% to -1.2%), 12.7% (-21.5% to -3.9%), and 6.7% (-11.0% to -2.4%), respectively. An IQR increase in urinary MDA was associated with a non-significant 4.6 % (-10.4% to 1.2%) reduction in C-ACT score for limitation of physical activities. In contrast, an IQR increase in FeNO (ppb) was associated with a non-significant increase in the individual score for limitation of physical activities by 9.9% (-0.4% to 20.1%). The associations between C-ACT scores and urinary 8-OHdG were inconclusive and non-significant.

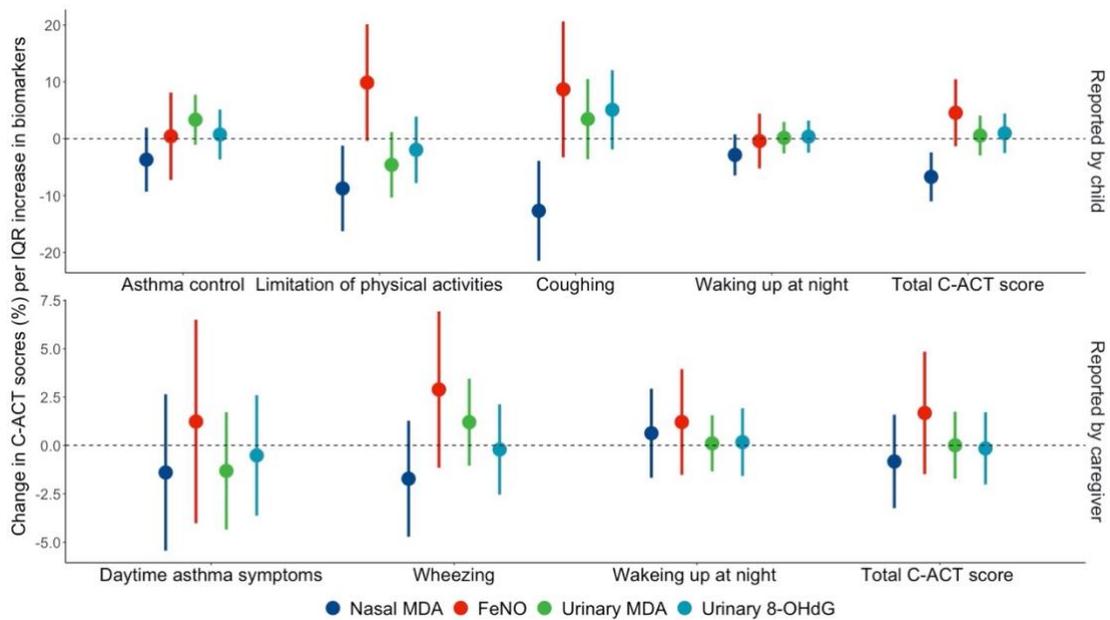


Figure 23. Estimated means and 95% confidence intervals for change in C-ACT scores (%) with one IQR increase in nasal MDA, FeNO, and urinary 8-OHdG.

5.2.3.4 Sensitivity analysis

We evaluated the robustness of the results through three sensitivity analyses. Firstly, we investigated whether the exposure-response relationships observed in the single-pollutant models can be retained after controlling for the co-pollutant. As shown

in Figure F3-F4, after adjusting for the co-pollutant, the exposure-response relationships were, for the vast majority of lag days, not markedly changed in terms of either effect size or statistical significance. We only found that the relationship of nasal MDA with personal O₃ exposure with lag 4 days changed from significant to non-significant. Secondly, we conducted the analysis excluding measurements from participants who suffered asthma exacerbation or used inhaled corticosteroids during the 2 weeks prior to the clinical visit. We did not find noticeable changes in the associations of pollution exposure with the biomarkers and C-ACT scores and the associations between the biomarkers and C-ACT scores in the sub-datasets (Figure F5-F10). Thirdly, we assessed the interaction of pollutant exposure with asthma exacerbation status, inhaled corticosteroids usage status, eosinophilic inflammation status, or sex in the LMER models examining the relationships of pollutant exposure and nasal MDA. The interactions were non-significant for both pollutants for the majority of the lag days (Table F4).

5.2.4 Discussion

The principal finding of this study is that personal PM_{2.5} and O₃ exposure measured several days prior were associated with increased nasal MDA concentration and worsened asthma symptom scores in asthmatic children. In addition, the increased nasal MDA concentration was associated with the worsened asthma symptom scores.

The results suggest that nasal MDA can be a useful biomarker for monitoring asthma worsening associated with PM_{2.5} and O₃ exposures.

We used a novel technology to collect nasal fluid using cellulose ester sampling strip. This method has been previously validated as a sensitive and reproducible approach to measure inflammatory cytokines in nasal fluid.²³⁰ In the present study, we firstly measured MDA in nasal fluid as a biomarker of oxidative stress in the upper respiratory tract and associated it with personal air pollutant exposure. The results showed that the increased day-to-day changes in PM_{2.5} and O₃ exposure were associated with significantly increased nasal MDA. The results were supported by previous *in vitro* and animal studies reporting that air pollutant exposure could increase the oxidative stress in human nasal epithelial cells and nasal mucosa of rats.^{35, 36} The present study, to the best of our knowledge, is the first to confirm the *in vitro* and *in vivo* animal study findings in humans in real-world settings.

As the first study investigating the effects of air pollution exposure on C-ACT scores, we found that the increases in day-to-day changes in PM_{2.5} and O₃ exposure were associated with decreased individual and total C-ACT scores both reported by children and their caregivers, indicating worsened asthma control status. The results further confirmed that air pollution exposure may lead to asthma aggravation; and it is important to reduce air pollution exposure as a strategy of the pediatric asthma management.²³⁷ In addition, we found that the increased nasal MDA was associated

with the worsened total asthma symptom scores and individual symptoms scores in coughing and limitation of physical activities reported by asthmatic children, while not with asthma symptom scores reported by caregivers. We speculate that the increased nasal burden of oxidative stress might have triggered an irritating sensation in the airway in the absence of airway inflammation (as inconclusive associations between C-ACT scores and FeNO were found), leading to coughing and limitation of physical activities. This is somewhat supported by our observation that the nasal MDA associations were with symptom scores reported by children but not by caregivers, because children should be more sensitive to their own sensory changes. Further studies are needed to understand the underlying biological mechanisms linking oxidative stress in the upper respiratory tract and changes in asthma symptoms.

ROS-induced oxidative stress in the respiratory tract may spill over to the circulatory system. Thus, we examined the associations of urinary MDA and 8-OHdG, as biomarkers of systemic oxidative stress, with air pollution exposure and C-ACT scores in asthmatic children. The associations of air pollution exposure with urinary MDA and 8-OHdG were non-significant and inconclusive in terms of effect size and statistical significance. The results were inconsistent with a previous study finding that air pollution exposure was associated with increased thiobarbituric acid-reactive substances, indicating increased systemic oxidative stress in children with asthma.²³⁸ The inconsistency might be attributed to the differences in demographic characteristics, air

pollution exposure assessment inaccuracy, and other unmeasured potential confounders. Similarly, we did not find clear and significant associations of biomarkers of systemic oxidative stress with asthma symptom scores, except for a negative association between urinary MDA and asthma symptom for physical activity limitation. The results were not in line with a previous study finding that the systemic oxidative stress of people with asthma were increased during asthma exacerbation.²³⁹ However, in this study, for 96% of the measurements, no asthma exacerbation was reported during the 2-week period prior to the clinical visits. We, hence, think that the symptoms in children with mild and moderate asthma, in the absence of asthma exacerbation, might not be sensitive enough to reflect the changes in systemic oxidative stress levels.

FeNO, as a biomarker widely used in asthma diagnosis and prognosis,^{225, 240} was associated with daily PM_{2.5} exposure in the present study, and the results are consistent with previous findings.³³ The results further confirmed that FeNO was a sensitive biomarker of air pollution exposure induced airway inflammation.²⁴¹ On the other hand, we did not find significant associations of FeNO with asthma symptom scores in asthmatic children, except for a positive association between FeNO and individual C-ACT score for physical activity limitation. The results were supported by a previous study reporting that there was non-significant difference in the proportion of C-ACT and FeNO in evaluating pediatric asthma control.²⁴² The results may be due to the large variability in FeNO level among asthmatic patients with different asthma heterogeneity

phenotypes, for which the clinical benchmarks have not been developed yet.²⁰⁴ Further research is needed to investigate the relationships of FeNO with asthma symptoms in different phenotypes.

Asthma exacerbation and inhaled corticosteroids usage may affect airway inflammation and oxidative stress in the respiratory tract and/or circulatory system. For this reason, we conducted sensitivity analyses removing participants who suffered an asthma exacerbation and used inhaled corticosteroids during the two weeks prior to the clinical visit. The results of this sub-set analysis and co-pollutant models did not change the findings from the main analysis (see Figure F3-F10). In addition, in considering the non-significant pollutant exposure by asthma exacerbation status, inhaled corticosteroids usage, eosinophilic inflammation status, or sex interaction (see Table F4), these four factors did not appear to modify the relationships of pollutant exposure and nasal MDA. On the other hand, the current study leveraged an air purification intervention to manipulate personal exposure to PM_{2.5} and O₃. The use of air purifiers might affect the exposure to gaseous pollutants, including volatile organic compounds and semi-volatile organic compounds. We were not able to assess personal exposure to these gaseous co-pollutants. Hence, a limitation of this study is that our findings on PM_{2.5} and O₃ effects may be subject to confounding by other co-pollutants.

5.2.5 Conclusion

The significant associations of nasal MDA with both air pollution exposure and asthma symptom scores indicated that air pollution exposure might lead to changes in oxidative stress in the nose and these changes were also related to asthma symptoms. The results confirm that oxidative stress, measured in the nasal fluid, is an important pathophysiologic pathway linking air pollution exposure and adverse respiratory health effects. These findings, along with the noninvasiveness of nasal fluid collection using a simple and inexpensive paper strip, suggest the potential importance of using nasal MDA as a biomarker for monitoring asthma status in relation to air pollutant exposures. The analysis of MDA can be readily and cost-effectively done in the lab using existing techniques, which makes nasal MDA a useful biomarker of oxidative stress in the upper respiratory tract. If this biomarker finds a wide application in the personalized asthma control management, it will motivate efforts to develop 'direct-read' technologies for measuring nasal MDA.

5.3 Part C: The Role of Endogenous Melatonin in Pathophysiologic and Oxidative Stress Responses to Personal Air pollutant Exposures in Asthmatic Children

The accompanying supporting information is included in Appendix G.

5.3.1 Introduction

Asthma is a common chronic and non-communicable disease in both children and adults.¹⁹ The prevalence of asthma is increasing in many regions of the world.²⁰ Exposure to air pollutants, including airborne fine particles (PM_{2.5}) and ozone (O₃), has often been associated with exacerbation of asthma.²²

Melatonin is a hormone endogenously excreted by the pineal gland with a marked circadian rhythm.³⁷ It is also a potent antioxidant and anti-inflammatory molecule that can suppress oxidative stress and inflammation in pulmonary and circulatory systems.^{65, 243} Melatonin can also inhibit mucus production by suppressing related gene expression, and it helps to improve asthma symptoms.⁶⁷ In addition, melatonin plays an important role in the inhibition of eosinophil peroxidase, which catalyzes the formation of oxidants and is a critical enzyme involved in the pathogenesis of asthma.⁶⁶ As the induction of oxidative stress and inflammation is an important pathophysiological pathway linking air pollution exposure and pulmonary dysfunction,^{22, 244} we hypothesized that melatonin plays an important role in oxidative stress and physiological responses to air pollution exposure in people with asthma.

To investigate this hypothesis, we leveraged the data collected from a cohort of 43 asthmatic children. We longitudinally measured multiple pulmonary health outcomes four times, including airway mechanics, lung function, and airway inflammation. Urinary 6-sulfatoxymelatonin (aMT6s) was measured as a surrogate of circulating melatonin.⁴⁰ We measured urinary malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) as two biomarkers of systemic oxidative stress.^{112, 114} We have previously examined the relationships of these pulmonary health outcomes and biomarkers of oxidative stress with personal exposures to PM_{2.5} and O₃.^{150, 245} In this study, we aim to explore whether endogenous melatonin affects asthmatic children's pulmonary response to air pollution exposure by examining the following relationships (1) pollutant exposures and urinary aMT6s, (2) urinary aMT6s and biomarkers of systemic oxidative stress, and (3) urinary aMT6s and indicators of pulmonary physiology.

5.3.2 Methods

5.3.2.1 Study participants

We recruited 43 children with mild or moderate asthma at the Shanghai General Hospital, located in Songjiang, a suburb of Shanghai, China. Each child had at least one episode of asthma exacerbation in the year preceding the study. Oral assent and written consent were obtained from all participants and their guardians, respectively. The study

protocol was approved by the Ethics Committee of Duke University Campus IRB and Shanghai General Hospital.

5.3.2.2 Study design

The detailed study design has been published previously.⁷² Briefly, this study leveraged data collected in a double-blind, randomized crossover study in which indoor PM_{2.5} and O₃ concentrations were manipulated through the operation of a portable air purifier (Atmosphere®, Amway, USA) in children's bedrooms for two weeks at a time for two distinct intervention periods – once with a fully functional purifier and once with only a coarse pre-filter included. The two intervention periods were separated by a two-week washout period. Each air purifier was equipped with a coarse filter, a high-efficiency particulate air (HEPA) filter, and an activated carbon filter to remove particulate matter and O₃. The operation schedule of the air purifier is shown in Figure G1. The impact of these interventions on indoor air quality has been previously reported.^{193-198, 229, 246} In the current study, the analysis is based on a panel study design in which children were clinically assessed four times. Specifically, there were two visits for each two-week intervention period, with one at the beginning and one at the end of the intervention. Efforts were made to conduct all measurements at the same time of day for all four visits from February 14 to April 14, 2017.

5.3.2.3 Air pollution exposure assessment

The concentration of PM_{2.5} and O₃ were simultaneously and continuously measured in children's bedrooms and outside a window of their homes. These pollutants were measured using a sensor box equipped with a Plantower PMS3003 sensor for PM_{2.5} and an Alphasense sensor (OX-A4) for O₃, and hourly averages of pollutant concentrations were generated. The sensors were field-calibrated before and after the study period and previously validated in various cities.^{194, 199-201} We also obtained hourly averages of ambient relative humidity, temperature, PM_{2.5}, and O₃ concentrations from the governmental environmental monitoring station closest to the research clinic (~9 km away) where children were assessed by a physician. In this study, we further calculated ambient air pollutant concentrations by averaging the concentrations measured by our outdoor sensors and the monitoring station to achieve a more precise estimation. Indoor/outdoor (I/O) ratios (Table G1) were used to calculate pollutant concentrations in different indoor microenvironments where no actual pollutant measurements were obtained; these ratios were based on the literature for similar building structures.^{71, 175} Combining the measured or calculated pollutant concentrations and detailed time-activity data, we calculated 24-hour average personal PM_{2.5} exposure and daily maximum 8-hour average personal O₃ exposure zero to five days (lag day 0-5) prior to each of the clinical visits. A detailed exposure assessment has been published previously.¹⁵⁰

5.3.2.4 Health outcomes

We measured the following pathophysiologic indicators of respiratory health. Fractional exhaled nitric oxide (FeNO), as a biomarker of pulmonary inflammation, using a NIOX VERO (Circassia Pharmaceuticals Inc., USA). Airway mechanics indicators were measured by impulse oscillometry (MasterScreen™ IOS, Becton, Dickinson and Company, Germany), including airway resistance at 5 Hz (R_5), resistance at 20 Hz (R_{20}), R_5 - R_{20} , impedance at 5 Hz (Z_5), reactance at 5 Hz (X_5), and resonant frequency (F_{res}). Increased R_5 , R_{20} , R_5 - R_{20} , and Z_5 reflect increased total airway resistance, large airway resistance, small airway resistance, and airway impedance, respectively. Decreased X_5 and increased F_{res} indicate worsened airway resilience. Pulmonary function was measured by spirometry (MasterScreen™ PFT system, Becton, Dickinson and Company, Germany), including forced expiratory volume in the first second (FEV₁), forced vital capacity (FVC), FEV₁/FVC ratio, peak expiratory flow (PEF), and forced expiratory flow at 25-75% of the FVC (FEF₂₅₋₇₅). Following standard clinical measurement procedures, we used the highest values among three consecutive airway mechanics and lung function measurements with variation smaller than 5%.

Urinary MDA was measured using a method previously described.¹¹² Urinary aMT6s and 8-OHdG were simultaneously measured using a HPLC-tandem mass spectrometry method previously reported.¹²¹

5.3.2.5 Statistical analysis

We report medians with interquartile range (IQR) and range for participants' baseline characteristics. First, we used linear mixed-effects regression (LMER) models to examine the relationships between the concentration of urinary aMT6s and air pollutant exposures. In these models, the urinary concentration of aMT6s was the dependent variable, and air pollutant exposure was the independent variable. We included fixed-effects for sex, age, ambient temperature and relative humidity averaged over the same time period as the pollutant exposure, upper respiratory tract infection-like symptoms, sleep duration, asthma exacerbation status, use of inhaled corticosteroids, use of supplements (i.e., vitamin, cod liver oil, and probiotics), dust allergy status, baseline eosinophil count, and travel status (whether or not the participant had traveled during the two weeks prior to each of the clinical visits). We included subject ID and the day of the week for the clinical visit as random-effect variables. From the model output, we calculated the percent change (and 95% confidence interval) of urinary aMT6s concentration associated with an IQR increase in air pollutant exposure.

Second, we used LMER models to examine the relationships between the concentration of urinary aMT6s and biomarkers of oxidative stress (i.e. urinary MDA and 8-OHdG). In these models, urinary concentration of aMT6s was the dependent variable, and the concentration of urinary MDA or 8-OHdG was the independent variable. We controlled for 24-hour average ambient temperature and relative humidity

and personal exposure to PM_{2.5} and O₃ at lag 0 along with the other fixed- and random-effects variables described in the first set of models. From the model output, we calculated the percent change (and 95% confidence interval) in the urinary aMT6s associated with an IQR increase in urinary MDA or 8-OHdG concentration.

Third, we used LMER models to examine the relationships between pulmonary health outcomes and the concentration of urinary aMT6s. In these models, each of the pulmonary health outcomes was the dependent variable, and the concentration of urinary aMT6s was the independent variable, along with the same covariate structure described in the second set of models. From the model output, we calculated the percent change (and 95% confidence interval) in the pulmonary health outcomes associated with per IQR increase in urinary aMT6s concentration.

Finally, we conducted two sensitivity analyses. First, we examined whether the results obtained in the single-pollutant models can be retained after controlling for the copollutant. Second, we further conducted separate analyses by excluding participants who had asthma exacerbation during the two weeks prior to each of the clinical visits (7 measurements from 6 subjects). All statistical analyses were conducted using *lme4* and *lmeTest* in R software (version 3.6.1).^{183, 184} A P-value of 0.05 was set as the cut point for statistical significance. A detailed description of equations used for the statistical models is provided in Supporting Information.

5.3.3 Results

5.3.3.1 Participant characteristics

Table 19 shows the baseline characteristics of participants. For the 43 children, the average age was 7.8 years and 17 (40%) were female. Of all of the clinic visits (n=172), 64 (37.2%) reported pulmonary infection-like symptoms, 64 (37.2%) used supplementation, 31 (18.0%) used inhaled corticosteroid, and 7 (4.1%) reported asthma exacerbation during the two weeks prior to each of the clinical visits. During the study period, the average (SD) sleep duration was 9.0 (0.8) hours per night. None of the participants reported using melatonin supplementation, and the urinary concentration of aMT6s measured in this study are within the range previously measured for healthy children aged 2.5 to 15.5 years.²⁴⁷

Table 19. Baseline characteristics of participants.

Subject Characteristics	Value
Age, mean \pm SD [range] (year)	7.8 \pm 2.3 [5-13]
Female, No. (%)	17 (40%)
Weight, mean \pm SD [range] (kg)	31.2 \pm 10.3 [19.0-59.0]
Height, mean \pm SD [range] (cm)	132.3 \pm 13.3 [110.0-166.0]
Dust allergy, No. (%)	10 (23%)
Blood eosinophil count, mean \pm SD [range] (/ μ L)	378.8 \pm 264.6 [80.0-1260.0]

Table 20. Statistical summaries of personal air pollutant exposure.

	Median [IQR]	Mean \pm SD	Range
PM_{2.5} ($\mu\text{g}/\text{m}^3$, 24-hour average)			
Lag 0	38.1 [24.8]	39.5 \pm 17.1	9.0 – 86.1
Lag 1	37.7 [27.2]	38.0 \pm 19.5	4.9 – 111.5
Lag 2	30.1 [31.0]	34.5 \pm 18.0	3.9 – 76.6
Lag 3	32.4 [22.2]	34.3 \pm 14.5	6.2 – 82.2
Lag 4	33.1 [27.2]	36.1 \pm 18.1	0.5 – 92.2
Lag 5	37.2 [33.5]	40.9 \pm 25.4	3.7 – 181.3
O₃ (ppb, maximum 8-hour average)			
Lag 0	20.8 [9.3]	21.8 \pm 8.5	6.1 – 52.9
Lag 1	18.4 [8.5]	18.9 \pm 7.1	5.4 – 46.1
Lag 2	19.1 [7.7]	19.6 \pm 6.5	5.6 – 51.5
Lag 3	20.2 [8.2]	20.9 \pm 7.0	6.3 – 51.1
Lag 4	19.6 [8.1]	21.4 \pm 8.2	1.1 – 56.4
Lag 5	22.1 [13.4]	24.4 \pm 10.2	8.5 – 52.3

5.3.3.2 Relationships between urinary aMT6s and air pollution exposure

The day-to-day personal air pollutant exposures are shown in Table 20, and we did not find strong Spearman correlations between personal PM_{2.5} and O₃ exposure (Table G2). Using the personal pollutant exposures and concentrations of aMT6s shown in Table 21, we examined the relationships between urinary aMT6s concentration and pollutant exposures. As shown in Figure 24, we found that an IQR increase in maximum daily 8-hour average O₃ personal exposure measured 1-, 2-, 3-, and 5-days prior to the clinic visits was associated with increased urinary aMT6s concentration by 23.0% (95% CI: -1.0% to 52.8%), 28.5% (6.6% to 54.9%), 30.5% (9.0% to 56.3%), and 40.7% (13.2% to 74.8%), respectively. In addition, we found that an IQR increase in 24-hour personal PM_{2.5} exposure measured 0-, 1-, and 2-days prior was associated with increased urinary aMT6s concentration by 17.0% (-1.9% to 39.5%), 16.8% (-3.8% to 41.8%), and 26.4% (0.3% to 59.3%), respectively.

Table 21. Pulmonary health outcomes and concentrations of urinary aMT6s, MDA, and 8-OHdG.

	Median [IQR]	Mean ± SD	Range
Melatonin			
aMT6s (ng/mg creatinine) *	3.1 [1.0]	3.2 ± 0.9	1.1 – 6.9
Airway Mechanics			
Z ₅ (cm H ₂ O/L/s)	8.9 [3.5]	8.9 ± 2.5	1.8 – 16.1
R ₅ (cm H ₂ O/L/s)	8.3 [3.2]	8.3 ± 2.5	-0.24 – 15.26
R ₂₀ (cm H ₂ O/L/s)	5.4 [2.1]	5.5 ± 1.5	1.65 – 11.1
R ₅ -R ₂₀ (cm H ₂ O/L/s)	2.8 [1.9]	2.9 ± 1.8	-4.6 – 9.5
X ₅ (cm H ₂ O/L/s)	-2.1 [1.9]	-2.0 ± 2.4	-12.1 – 8.2
Fres (Hz)	21.1 [5.8]	19.1 ± 6.3	3.2 – 32.2
Lung Function			
FEV ₁ (L)	1.6 [0.7]	1.7 ± 0.5	0.9 – 3.3
FVC (L)	1.9 [0.8]	2.1 ± 0.6	1.1 – 4.4
FEV ₁ /FVC (%)	84.8 [11.7]	83.1 ± 8.1	60.8 – 97.6
PEF (L/s)	1.6 [0.8]	1.7 ± 0.6	0.6 – 3.8
FEF ₂₅₋₇₅ (L/s)	3.6 [1.6]	3.9 ± 1.1	1.8 – 7.2
Airway Inflammation			
FeNO (ppb)	17.0 [21.0]	23.6 ± 20.1	5.0 - 120
Oxidative Stress			
MDA (ng/mg creatinine) *	6.0 [0.9]	6.1 ± 0.8	4.3 – 10.0
8-OHdG (ng/mg creatinine) *	0.6 [2.7]	0.5 ± 2.2	-4.5 – 6.4

* natural logarithm transformed

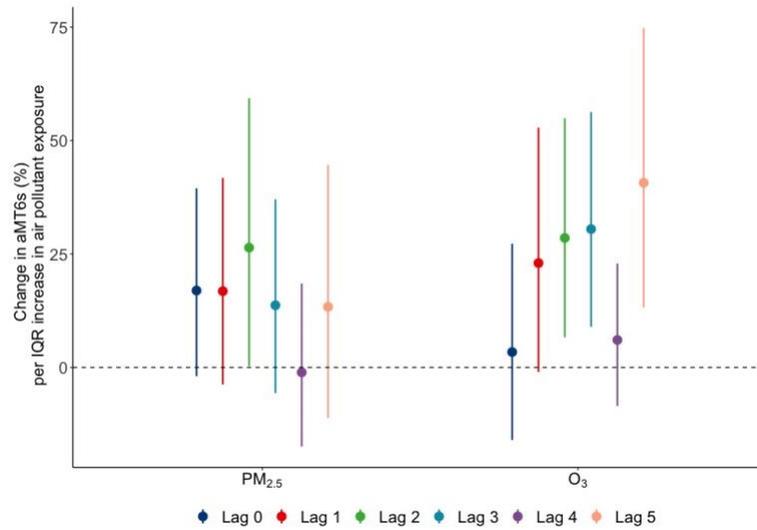


Figure 24. Change in urinary aMT6s (%) for an IQR increase in 24-hour average PM_{2.5} exposure and maximum daily 8-hour average O₃ personal exposure.

5.3.3.3 Relationships of urinary aMT6s with urinary MDA and 8-OHdG

Using the concentrations of aMT6s, MDA, and 8-OHdG shown in Table 21, we examined the relationships of urinary aMT6s with urinary MDA and 8-OHdG concentration. As shown in Figure 25, we found that an IQR increase in urinary MDA and 8-OHdG concentrations were associated with significantly increased urinary aMT6s concentrations by 73.4% (52.6% to 97.0%) and 41.7% (22.8% to 63.4%), respectively.

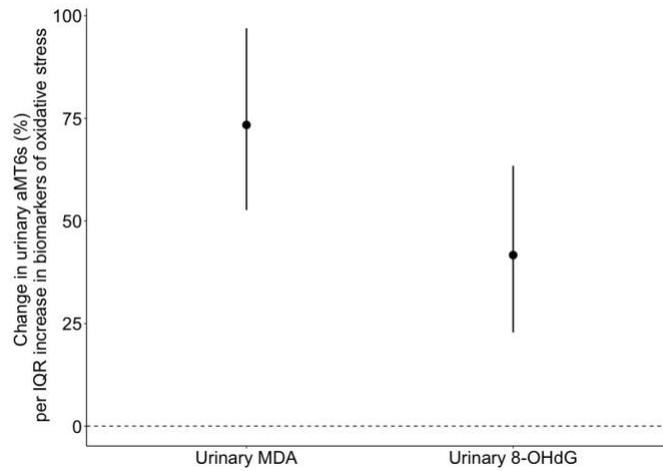


Figure 25. Estimated means and 95% confidence intervals for change in urinary aMT6s (%) for an IQR increase in urinary MDA or 8-OHdG.

5.3.3.4 Relationships between aMT6s and pulmonary health outcomes

Using the concentrations of aMT6s and pulmonary health outcomes shown in Table 21, we examined the relationships between pulmonary health outcomes and urinary aMT6s concentration. As shown in Figure 26, we found that an IQR increase in urinary aMT6s concentration was associated with significantly decreased F_{res} and FeNO by 8.1% (-14.3% to -1.8%) and 8.1% (-15.5% to -0.1%), respectively.

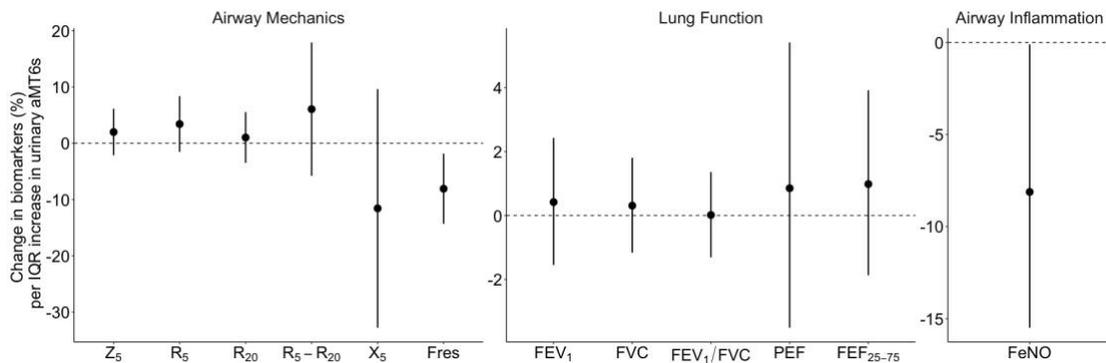


Figure 26. Estimated means and 95% confidence intervals for change in pulmonary health outcomes (%) one IQR increase in urinary aMT6s.

5.3.4 Discussion

As a potent antioxidant and anti-inflammatory molecule, melatonin generated endogenously may play a role in pathophysiologic responses to air pollution exposure. The main findings of this study include: (1) that urinary MDA or 8-OHdG concentrations were significantly and positively associated with urinary aMT6s concentrations; (2) that increases in daily personal exposure to O₃ and PM_{2.5} were significantly associated with increased urinary aMT6s concentrations; and (3) that increased urinary concentration of aMT6s was associated with decreased airway inflammation (FeNO) and improved airway resilience (Fres). Taking these results together with our previous finding that increasing exposure to PM_{2.5} and to O₃ was each associated with increased systemic oxidative stress in the same study subjects,²⁴⁵ we present evidence to support a novel biological mechanism that air pollution exposure-induced reactive oxygen species (ROS) may stimulate the excretion of melatonin, which may be beneficial to pulmonary health because melatonin can suppress airway inflammation and improve airway resilience.

This study found that an IQR increase in urinary MDA and 8-OHdG concentration was associated with significantly increased urinary aMT6s concentration by 73.4% (52.6% to 97.0%) and 41.7% (22.8% to 63.4%), respectively. The results were consistent with our previous study reported that an IQR increase in urinary MDA and 8-OHdG concentration was associated with significantly increased urinary aMT6s

concentration by 9.3% (3.4% to 15.6%) and 17.4% (10.3% to 25.0%), respectively, in healthy adults, aged 19-52 years old.¹⁵⁴ These positive associations could be explained by a recent study reporting that melatonin could be synthesized in mitochondria in response to the increases in oxidative stress at the cellular level.¹²⁵ It could also be an important mechanism that leverages melatonin as a key endogenous factor in limiting oxidative damages.⁵³ On the other hand, the effect sizes of these associations are greater in asthmatic children than those in healthy adults. The results suggest a stronger ROS-induced stimulation of melatonin secretion in asthmatic children than in healthy adults. The underlying mechanisms need to be further investigated.

In this study, we found that short-term (Lag 0 or Lag 1) exposure to air pollutants was not significantly associated with urinary aMT6s concentrations in asthmatic children (see Figure 24). The results were supported by our previous study finding nonsignificant associations of urinary aMT6s concentrations with 12-hour and 24-hour average personal air pollutant exposures (PM_{2.5}, O₃, and NO₂) in healthy adults.¹⁵⁴ A potential explanation is that ROS induced by short-term air pollution exposure may neutralize circulating melatonin and stimulate melatonin excretion, leading to a net nonsignificant change in circulating melatonin.

In contrast, we found that PM_{2.5} exposure measured 2 days prior and O₃ exposure measured 2, 3, and 5 days prior were associated with significantly increased urinary aMT6s concentration. The results suggest that it may take a longer time for the

air pollution-induced stimulation of melatonin to exceed the amount of melatonin that is neutralized. The result could be partially supported by our previous finding that a 2-week average PM_{2.5} and SO₂ exposure are significantly and positively associated with urinary aMT6s concentration in healthy adults.²³⁴ However, in the same study, we also found that 2-week average O₃ exposure was negatively associated with urinary aMT6s. This inconsistency might be attributed to the strong negative correlations of 2-week O₃ exposure with 2-week NO₂ (R²=0.85) and SO₂ (R²=0.79) exposures and the difference in the age (children versus adults) and disease status (asthmatic versus healthy) of the study participants. Future studies are suggested to further investigate the effects of long-term air pollution exposure on circulating melatonin level considering a design with a larger sample size and well-controlled co-pollutant exposures.

In this study, we found that increasing aMT6s concentrations were significantly associated with decreasing FeNO and Fres values. To the best of our knowledge, this is the first study in humans to investigate the relationships of endogenous melatonin with FeNO and airway mechanics. The present results could be partially supported by the findings in mice with ovalbumin-induced allergic asthma.²⁴⁸ The rodent study showed that melatonin administration (10-15 mg/kg) significantly attenuated airway inflammation and hyperresponsiveness, decreased the number of inflammatory cells and inflammatory cytokines, reduced the mucus production in lung tissue, and largely suppressed the expression and activity of matrix metalloproteinase 9 (MMP-9).²⁴⁸ It has

been reported that MMP-9 mediates airway remodeling by regulating the infiltration of inflammatory cells.²⁴⁹ The overexpression of MMP-9 could increase T-helper type 2 cytokine levels,²⁵⁰ which have been widely associated with the activation and maturation of eosinophils, leading to the aggravation of the asthmatic responses.²⁵¹ Increased MMP-9 level in lung tissue has been found in people with asthma and a murine asthma model.^{252, 253} These previous results suggest that melatonin could attenuate airway inflammation and improve airway mechanics by suppressing the expression of MMP-9 and reducing inflammatory molecules. Hence, the results of the present study further suggest that ROS-stimulated melatonin may serve as a defense mechanism to alleviate the adverse effects of air pollution on airway resilience and inflammation in children with asthma. In spite of this defense mechanism, there was a net adverse effect of air pollution exposure on airway inflammation, illustrated by our previous finding that increased personal exposures to PM_{2.5} and O₃ were associated with increased FeNO in the same study subjects.¹⁵⁰

On the other hand, we found nonsignificant relationships of urinary aMT6s with lung function, airway resistance, and airway reactance. The results were inconsistent with a previous study reporting that increased circulating melatonin levels were significantly associated with decreased FEV₁ in patients with nocturnal asthma.⁶⁸ In the same study, the authors also reported that peak circulating melatonin levels in patients with nocturnal asthma were significantly higher than those of the healthy controls. The

authors hypothesized that this adverse pulmonary health effect might be attributed to the melatonin's effects on the augmentation of peripheral blood mononuclear cell production of pro-inflammatory cytokines, including Interleukin-1, Interleukin-6, and Tumor necrosis factor- α .²⁵⁴ However, no further studies have been done to test this hypothesis. The discrepancy between the previous and the current study might be attributed to different asthma phenotypes and other uncontrolled confounders.

Concerning the adverse relationships between circulating melatonin and lung function examined in some of the previous observational studies, the use of melatonin supplementation as a sleep promoter is not recommended for asthmatic patients.^{65, 68} One study, however, found that following oral supplementation of 3 mg melatonin 2 hours before bedtime for 28 days, people with mild or moderate asthma reported improved sleep quality, albeit without significant changes in asthma symptoms, pulmonary function, and asthma medication usage.²⁵⁵ In contrast, in this study, we found that increasing levels of endogenous melatonin were significantly associated with reduced airway inflammation and improved airway resilience. Concerning the established understanding of the beneficial effects of melatonin on neutralizing ROS, suppressing inflammation, and improving sleep quality, we argue that the use of melatonin supplementation or treatment in people with asthma is worth further investigation. It is premature to discourage or recommend the use of melatonin supplementation based on the limited body of literature on this topic.

A limitation of this study is the lack of direct measurement of light exposure, which affects circulating melatonin levels. However, the following features of the study design and data analysis help minimize the influence of this limitation on the main findings. First, we adjusted for sleep duration in our LMER models to control for potential light exposure. Second, to take into account diurnal variation in circulating melatonin levels, we tried to collect urine samples at the same time of day for all four visits. Third, we controlled ambient temperature and relative humidity averaged over the past day, reflecting outdoor weather as a proxy for outdoor light exposure. Fourth, we used linear mixed-effects models with subject ID as the random effect to control the potential differential average light exposure of different subjects.

The robustness of the main results was evaluated through two sensitivity analyses. First, after adjusting for the copollutant, the exposure–response relationships between air pollutant exposures and urinary aMT6s concentration were, for the vast majority of lag days, not markedly changed in terms of both statistical significance and effect size (Figure G2). As asthma exacerbation largely affects the pulmonary health system, we conducted sensitivity analyses on a subset of the measurements, excluding, on a visit-by-visit basis, measurements of participants who had asthma exacerbation during the two weeks prior to each clinical visit (7 measurements from 6 participants were excluded). The results show no marked difference from the results of the main

analyses, in terms of both statistical significance and effect size (Figure G3-G5), supporting the robustness of our main findings.

5.3.5 Conclusion

Within the natural range of aMT6s concentrations, increasing urinary MDA or 8-OHdG concentration was associated with increased urinary aMT6s concentrations in asthmatic children. The result suggests that increasing systemic oxidative stress levels stimulate melatonin excretion. We found that increases in daily personal exposure to O₃ and PM_{2.5} measured 2-5 days prior were associated with increased urinary aMT6s concentrations. We also found that increased concentration of urinary aMT6s was associated with improved pulmonary inflammation (reduced FeNO) and airway resilience (decreased Fres). Taking these results together with our previous findings that increased exposure to O₃ or PM_{2.5} was associated with worsened airway mechanics and inflammation, increased systemic oxidative stress may stimulate the excretion of melatonin as a defense mechanism to alleviate the adverse effects of air pollution exposure.

Chapter 6. Conclusions

6.1 Conclusions

One major pathophysiologic pathway linking air pollutant exposures and adverse health outcomes is the induction of reactive oxygen species (ROS) and inflammation by inhaled pollutants. Melatonin is an important hormone excreted by the pineal gland with a marked circadian rhythm.³⁷ Melatonin can reduce oxidative stress by directly neutralizing ROS, stimulating antioxidant enzymes, and suppressing the activity of pro-oxidant enzymes.⁵³ In addition, melatonin can modulate inflammatory responses. Concerning oxidative stress and inflammation in the pulmonary system are critical factors in the pathogenesis of respiratory diseases, melatonin may also play an important role in respiratory health.⁶⁵

This dissertation research aims to investigate the role of melatonin in pathophysiological responses to air pollution exposure. It focuses on melatonin's effects on the oxidative, inflammatory, and physiological responses to air pollution exposure.

In Aim 1 of this dissertation, an analytical method using HPLC-MS to simultaneously measure urinary aMT6s, a major metabolite of melatonin, and 8-OHdG, a product of DNA oxidative damage, was developed. Compared with conventional methods, this new method reduced sample treatment time and cost by replacing a multi-step solid phase extraction procedure with a liquid-liquid extraction procedure. In addition, this new method has adequate precision, accuracy, and sensitivity to measure

both urinary 8-OHdG and aMT6s, a major metabolite of melatonin and a well-established surrogate of circulating melatonin.

In Aim 2 of this dissertation, the effects of melatonin on the systemic oxidative responses to air pollution exposure in healthy adults were investigated. Urinary aMT6s concentration was significantly and positively associated with urinary MDA and 8-OHdG concentrations. A potential hypothesis is that cellular oxidative stress may stimulate the production of melatonin in the cells. I also found that natural variation in urinary aMT6s concentration is a potential confounder for the relationships between short-term air pollution exposure (12-hour) and biomarkers of oxidative stress.

Within the Aim 3 of this dissertation, the effects of melatonin on the inflammatory responses to air pollution exposure in healthy adults were investigated.

Aim 3 comprises of Part A and Part B.

In Part A of Aim 3, the relationships between air pollution exposures and inflammatory cytokines were examined. The exposure-response analyses showed that O₃ exposure was significantly associated with pro-inflammatory cytokines, and these associations were affected by exposure time durations. Specifically, shorter-term O₃ exposure (12-hour) was associated with decreased concentrations of pro-inflammatory cytokines. However, longer-term O₃ exposure was associated with increased concentrations of pro-inflammatory cytokines. The relationships between inflammatory cytokines with PM_{2.5}, NO₂, and SO₂ exposures were nonsignificant and unclear.

In Part B of Aim 3, the role of melatonin in inflammatory responses to air pollution exposure was investigated. I found significant and negative associations between urinary aMT6s concentration and plasma pro-inflammatory cytokines and between 2-week O₃ exposure and urinary aMT6s concentration. The results of mediation analyses indicated that urinary aMT6s could be a potential mediator for the associations between 2-week O₃ exposure and pro-inflammatory cytokines examined in Part A of Aim 3.

Within the Aim 4 of this dissertation, the role of melatonin in the physiological and oxidative responses to air pollution exposure in asthmatic children was investigated. Lung function and airway inflammation (FeNO) were measured as conventional indicators of airway physiology. In addition to that, airway mechanics were measured to indicate physiological changes in airways, especially in small airways.

In Part A of Aim 4, the relationships of pollution exposures and pulmonary physiology indicators were examined. Day-to-day changes in personal exposure to PM_{2.5} was associated with worsened airway inflammation and small and total airway resistance. These associations were not affected by the co-presence of O₃ at low levels when O₃ itself was not associated with any of the pulmonary outcomes.

In Part B of Aim 4, I investigated whether MDA in the nasal fluid could be useful in asthma control in relation to air pollution exposure. The significant associations of nasal MDA with both air pollution exposure and asthma symptom scores indicated that

air pollution exposure might lead to changes in oxidative stress in the nose, and these changes were also related to asthma symptoms. The results confirm that oxidative stress, measured in the nasal fluid, is an important pathophysiologic pathway linking air pollution exposure and adverse respiratory health effects.

To understand the role of melatonin in physiological responses to air pollution exposure, Part C of Aim 4 investigates the relationships of urinary aMT6s with personal air pollutant exposure, indicators of pulmonary physiology, and biomarkers of oxidative stress. The results found that increased urinary MDA or 8-OHdG concentration was associated with increased urinary aMT6s concentration in asthmatic children. The result suggests that increasing oxidative stress levels in the circulatory system may stimulate melatonin excretion. We found that an increase in daily personal exposure to O₃ and PM_{2.5} measured 2-5 days prior to the urine collection were associated with increased urinary aMT6s concentration. We also found that increased concentration of urinary aMT6s was associated with improved pulmonary inflammation (reduced FeNO) and airway resilience (decreased Fres). Taken these results together with our previously finding that increased exposure to O₃ or PM_{2.5} was associated with worsened airway mechanics and inflammation, increased systemic oxidative stress may stimulate the excretion of melatonin as a defense mechanism to alleviate the adverse effects of air pollution exposure.

Taken together all these results, this dissertation research finds that natural variation in circulating melatonin levels is a potential confounder for the relationships of short-term (12-hour) air pollution exposure (PM_{2.5}, O₃, and NO₂) with biomarkers of oxidative stress and a potential mediator for the relationships between 2-week O₃ exposure and pro-inflammatory cytokines. Moreover, this research suggests that ROS-stimulated melatonin may serve as a defense mechanism to alleviate the adverse effects of air pollution on airway resilience and inflammation in children with asthma. The results point to the importance of considering the effects of endogenous melatonin in future studies involving oxidative, inflammatory, and physiological responses to air pollution exposure. In addition, this dissertation indicates that airway mechanics and nasal MDA could be useful biomarkers in asthma management. Given the small sample size (43 children with asthma) of the study evaluated in this dissertation, the applications of these biomarkers in asthma management should be further investigated in a larger study.

6.2 Implications and future research directions

6.2.1 Aim 1

Melatonin has been widely reported to neutralize ROS and plays a crucial role in repairing damaged DNA segments.¹¹⁹ The excretion of 8-OHdG molecules may be decreased due to the diminished amount of melatonin to help repair ROS-DNA adducts.

This relationship is supported by a previous study finding that night-shift workers have both lower urinary 8-OHdG concentration and circulating melatonin levels compared to day-shift workers.¹²⁰ Considering the potential effects of melatonin (the parent compound of aMT6s) on 8-OHdG, it is useful to simultaneously measure these two compounds in future environmental health studies involving DNA damage repair and oxidative stress-related pathophysiological pathways.

6.2.2 Aim 2

Air pollutant exposures have been often associated with biomarkers of oxidative damage, while generating somewhat inconsistent findings. This inconsistency may be attributed to different demographic characteristics, exposure inaccuracy, and other potential confounders. Concerning that melatonin plays a vital role in modulating oxidative stress, Aim 2 of this dissertation research explores whether this inconsistency is partly due to the confounding by natural variation in circulating melatonin levels.

One finding of Aim 2 indicates that short-term air pollution exposure (12-hour) is significantly and negatively associated with urinary aMT6s concentration. It is mainly because compared to the day time level, circulating melatonin level is higher during the night time, while the ambient air pollution level is lower during the night time. After adjusting for the collection time in the LMER model, the relationships change to be nonsignificant. The results suggest that short-term air pollution exposure in this study cannot induce enough ROS to significantly affect circulating melatonin. More studies are

recommended to investigate whether acute and higher-level air pollution exposure may affect circulating melatonin levels and/or affect melatonin metabolism.

In Aim 2, urinary aMT6s concentration is significantly and positively associated with urinary MDA and 8-OHdG in healthy adults. The current results can be partially explained by a recent finding that melatonin could be synthesized in mitochondria in response to the increase in oxidative stress.¹²⁵ Therefore, increased ROS levels may stimulate melatonin synthesis to neutralize ROS, which could be a protective mechanism to reduce oxidative damages. However, this stimulation process is still poorly understood, and many questions that need to be answered, including: (1) In which type of cells or organs does this stimulation take place? (2) What mediators are involved in this stimulation process? (3) What is the role of this stimulation process in body anti-oxidative systems?

The result of Aim 2 further indicates that the natural variation in circulating melatonin does not modify the relationships between short-term air pollution exposures and biomarkers of oxidative stress. Due to the limited sample size and a relatively narrow range of melatonin levels, it should be cautious of drawing any conclusions from the current study results. Concerning taking melatonin supplementation would largely increase circulating melatonin level,⁴¹ future studies are needed to investigate whether it is an effect modifier for the relationships of air pollution exposure and oxidative stress.

As a major finding of Aim 2, natural variation in circulating melatonin has been found as a potential confounder for the relationships between short-term (12-hour) air pollution exposures and biomarkers of oxidative stress. The result suggests the importance of adjusting for circulating melatonin levels in future studies investigating the effects of short-term (< 24 hours) ambient air pollution exposure on systemic oxidative stress. If the melatonin measurement is not feasible, it is recommended to collect all biospecimen at the same time of a day to minimize the impacts of the natural variation in melatonin.

6.2.3 Aim 3

Melatonin may have played a role in inflammatory responses to air pollutant exposures. The Aim 3 of this dissertation research explores the relationship between air pollution exposure and inflammatory responses (Part A) and how melatonin affected this relationship (Part B).

6.2.3.1 Part A

In Part A of Aim 3, the results indicate that exposure to O₃ for different time durations affect inflammatory responses differently. Specifically, a shorter-term (12-h) exposure to O₃ is negatively associated with proinflammatory cytokine levels. It suggests that acute O₃ exposure may stimulate the production of antioxidant enzymes as a compensatory mechanism. However, other studies also reported that acute exposure to O₃ at a higher level than the current study was associated with increased

proinflammatory responses. These results indicate that the health effects of acute O₃ exposure are largely affected by exposure levels. In contrast, a longer-term (2-week) O₃ exposure is positively associated with proinflammatory cytokines. It confirms previous findings that persistent O₃ exposure can upregulate the redox homeostasis towards an enhanced proinflammatory status.

These mechanistic insights can help develop therapeutic and/or preventive strategies to reduce the adverse effects of O₃ exposure. To approach this goal, further studies are needed to examine the oxidative and inflammatory responses to O₃ exposure with a wider range and in more susceptible individuals, including people with asthma.

6.2.3.2 Part B

In the Part B of Aim 3, the results show a significant and negative association between 2-week O₃ exposure and urinary aMT6s concentration. It suggests that increased 2-week O₃ exposure may shift the redox homeostasis towards a higher oxidation status, and it may enhance the consumption of melatonin as an antioxidant. As a major finding in Aim 2, an excessive amount of oxidative stress may stimulate the synthesis of melatonin. However, this stimulation effect was not seen in response to O₃ exposure. More studies are needed to further investigate the underlying mechanisms.

On the other hand, in this study, significant and positive associations are found of aMT6s with PM_{2.5} and SO₂. A potential explanation is the strong negative associations of 2-week O₃ exposure with PM_{2.5} and SO₂ exposure. Therefore, to confirm and expand

the current findings, future studies are needed to consider a design with well-controlled co-pollutant exposure, longer pollutant exposure duration, and a larger sample size.

The significant and negative associations between the concentration of urinary aMT6s and blood pro-inflammatory cytokines confirm the anti-inflammatory property of melatonin. In contrast, other *in vitro* and animal studies also reported that melatonin might display pro-inflammatory properties, including the augmentation of peripheral blood mononuclear cells production of pro-inflammatory cytokines.²⁵⁴ However, it is still unknown whether and how melatonin enhances pro-inflammatory responses in humans.

Part B's major finding indicates that melatonin is a potential mediator for the relationship between 2-weeks O₃ exposure and pro-inflammatory cytokines. The results suggest that pro-inflammatory response to ozone exposure in the preceding two weeks may partly due to the depletion of endogenous melatonin by O₃ exposure. Further studies are recommended to investigate whether taking melatonin supplementation would help decrease the inflammatory responses to O₃ exposure.

6.2.4 Aim 4

The Aim 4 of this dissertation research explores the relationship between air pollution exposure and indicators of pulmonary physiology in asthmatic children (Part A); the effectiveness of nasal MDA for monitoring asthma control in relation to air

pollution exposure (Part B); and the role of melatonin in physiological responses to air pollution exposures (Part C).

6.2.4.1 Part A

The main finding of Part A is that day-to-day changes in personal PM_{2.5} exposure are associated with increased airway resistance and inflammation. In contrast, the relationships between pollutant exposures and spirometry lung function are not significant and clear. The results further confirm that airway mechanics could reflect early-stage and subtle changes in pulmonary physiology in responses to air pollution exposure compared to lung function. As improving airflow is a key challenge in asthma management, these associations indicate the importance of reducing personal exposure to PM_{2.5} as a practical means of asthma control, especially for those living in places with high ambient and/or indoor PM_{2.5} concentrations.

6.2.4.1 Part B

In Part B of Aim 4, nasal MDA has been associated with air pollutant exposures (PM_{2.5} and O₃) and asthma symptom scores. These findings, along with the noninvasiveness of nasal fluid collection using a simple and inexpensive paper strip, suggest the potential importance of using nasal MDA as a biomarker for monitoring asthma status in relation to air pollutant exposures. If this biomarker finds a wider application in the personalized asthma control management, it will motivate efforts to develop “direct-read” technologies for measuring nasal MDA. Given that nose is a

prime portal of entry for air pollutants into the human body, nasal fluid could be an important biospecimen containing important biomarkers, other than MDA, that could indicate many pathophysiologic processes. Future studies could investigate the relationships between inflammatory cytokines in nasal fluid and air pollution exposure.

6.2.4.1 Part C

One of the major findings in Part C is the significant and positive associations of urinary aMT6s with urinary MDA and 8-OHdG in asthmatic children. The effect sizes of these associations are much larger than those found in healthy adults (Aim 2). The results suggest a more vital ROS-induced stimulation of melatonin synthesis in asthmatic children. However, the underlying mechanics are still unclear. Whether this stronger effect is related to a younger age, asthma, or both need to be further investigated.

As an antioxidant, melatonin can be neutralized by increased ROS induced by air pollution exposure. However, this study found positive relationships between air pollutant exposures and urinary aMT6s. The results suggest that more melatonin has been stimulated than those have been neutralized leading to a net increase in circulating melatonin level. The increased melatonin level may further help to decrease airway inflammation and improve airway resilience. The protective role of melatonin in physiological responses to air pollution exposures and the underlying biological mechanisms need to be further investigated.

The therapeutic potential of melatonin in asthma treatment has been widely reported. However, taking melatonin supplementation has not been suggested due to the previous observational study finding adverse associations between circulating melatonin levels and lung function.⁶⁸ In contrast, in this study, we found that increasing levels of endogenous melatonin were significantly associated with reduced airway inflammation and improved airway resilience. In addition, concerning the favorable effects of melatonin on neutralizing oxidative stress, suppressing inflammation, and improving sleep quality, the use of melatonin administration in asthma treatment is worthy of further investigation.

6.2.5 Overall recommendations

The findings from this research suggest that the role of endogenous melatonin in the health effects of air pollution should be considered. When we move towards personal level protection of air pollution exposure, it is imperative to understand individual differences in physiological and pathological responses to air pollution exposure. The present findings support that endogenous melatonin can modulate oxidative, inflammatory, and physiological responses to air pollution exposure in a beneficial way. This work supports the need for future trials to assess the efficacy and effectiveness of melatonin supplementation to mitigate the adverse health effects of air pollution exposure at the individual level. This is particularly important for susceptible population living in highly polluted areas (e.g., developing countries and areas subject

to frequent wildfires), people having melatonin deficiency, and those using dirty household fuels.

Appendix A

Appendix A contains the supporting information for Chapter 2, published online from He, L.; Liu, X.L.; Zhang, J.J. Simultaneous quantification of urinary 6-sulfatoxymelatonin and 8-hydroxy-2'-deoxyguanosine using liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B*. 2018, 1095, 119-126 (Publisher: Elsevier).

Table A1. Instrumental responses for aMT6s, 8-OHdG, and ¹⁵N5-8-OHdG in relation to extraction solvent: the same amount of standards were spiked. Results show that among three organic solvents, methanol extraction generated highest instrumental response signal.

Area under peak	Methanol (n=3)	Acetonitrile (n=3)	Isopropyl alcohol (n=3)
aMT6s	419,115	364,244	148,958
8-OHdG	37,737	7,171	4,986
¹⁵ N5-8-OHdG	10,567	1,780	6,885

Table A2. Instrumental responses for aMT6s, 8-OHdG, and ¹⁵N5-8-OHdG in relation to methanol concentrations used in extraction: Results show that 20% of methanol generated the highest instrumental response signal.

Area under peak	Methanol (80%)	Methanol (60%)	Methanol (40%)	Methanol (20%)
aMT6s	19,659	21,843	38,686	122,261
8-OHdG	6,904	6,157	14,970	43,007
¹⁵ N5-8-OHdG	261	1,952	7,579	18,056

Table A3. Instrumental responses for aMT6s, 8-OHdG, and ¹⁵N5-8-OHdG in relation to NaOH concentrations used in extraction along with 20% methanol: Results show that adding 0.5% of NaOH generated the highest instrumental response signal for 8-OHdG and ¹⁵N5-8-OHdG and the second highest response signal for aMT6s.

Area under peak	Acetate	0.5% 1M NaOH	1% 1M NaOH	3% 1M NaOH	10% 1M NaOH	20% 1M NaOH
aMT6s	170,175	133,259	68,081	93,994	49,800	39,875
8-OHdG	6,904	25,496	18,724	14,113	8,104	2,271
¹⁵ N5-8-OHdG	2,415	9,706	6,629	7,846	2,787	1,829

Table A4. Relative standard deviation (RSD) values from triplicate analyses of urine samples doped with different amount of 8-OHdG and aMT6s standards: Results show that RSD values decreased as analyte concentrations increased.

	RSD (8-OHdG)	RSD (aMT6s)
S1 (25 ng/mL, n=3)	23.3%	7.2%
S2 (50 ng/mL, n=3)	2.1%	5.9%
S3 (100 ng/mL, n=3)	9.1%	3.8%

Appendix B

Appendix B contains the supporting information for Chapter 3, published online from He, L.; Cui, X.; Xia, Q.; Li, F.; Mo, J.; Gong, J.; Zhang, Y.; Zhang, J. J., Effects of personal air pollutant exposure on oxidative stress: Potential confounding by natural variation in melatonin levels. *International journal of hygiene and environmental health*. 2020, 223, (1), 116-123 (Publisher: Elsevier).

B1. Statistical Analysis

B1.1 Association between urinary aMT6s with 8-OHdG and MDA

Linear mixed-effects regression (LMER) models were constructed to determine the association between aMT6s with 8-OHdG and MDA following *Formula B1* using the *lme4* and *lmeTest* packages of the R software (version 3.3.2). The dependent variable in the model is the concentration of 8-OHdG or MDA, and the independent variable is the concentration of aMT6s. All biomarkers were natural logarithm-transformed to meet the assumptions of linear mixed-effects regression. The models were adjusted for fixed-effect covariates including menstruation status, smoking status, study factor, respiratory infection status, age, sex, and 24h average temperature & relative humidity. An individual-specific random intercept was added to account for correlation within the same participant. The day of week that urine collected was controlled as another random effect.

Formula B1

$$Y_{ij} \sim \beta_0 + \beta_1 aMT6s_{ij} + \beta_2 RH_{ij} + \beta_3 Temp_{ij} + \beta_4 Menses_{ij} + \beta_5 Smoking_i + \beta_6 Study_i + \beta_7 Fever_{ij} + \beta_8 Sex_i + \beta_9 Age_i + P_i + W_{ij} + \epsilon$$

B1.2 Dose-response relationships

B1.2.1 The association between air pollutant exposure and aMT6s

LMER models were constructed to determine the relationship between air pollutant exposure and aMT6s following *Formula B2*. The dependent variable in the model is the concentration of aMT6s, and it was natural logarithm-transformed. The independent variable is 12h or 24h average air pollutant exposure prior to sample collection. In addition to the fixed-effect covariates described above, time of urine collection was added as another covariate. The two-pollutant model was developed based on the single-pollutant model as following *Formula B3*.

B1.2.2 The association between air pollutant exposure and 8-OHdG and MDA without controlling for aMT6s

LMER models were constructed to determine the relationship between air pollutant exposure and 8-OHdG and MDA following *Formula B2*. The dependent variable is the concentration of 8-OHdG and MDA with natural logarithm-transformation. The independent variable is 12h or 24h air pollutant exposure. Fixed effects and random effects were described B1.2.1. The two-pollutant model was shown as following *Formula B3*.

Formula B2

$$aMT6s_{ij} \sim \beta_0 + \beta_1 Pol_{ij} + \beta_2 RH_{ij} + \beta_3 Temp_{ij} + \beta_4 Smoking_i + \beta_5 Menses_{ij} + \beta_6 Study_i \\ + \beta_7 Col_time_{ij} + \beta_8 Fever_{ij} + \beta_9 Sex_i + \beta_{10} Age_i + P_i + W_{ij} + \varepsilon$$

Formula B3

$$aMT6s_{ij} \sim \beta_0 + \beta_1 Pol_{ij} + \beta_2 RH_{ij} + \beta_3 Temp_{ij} + \beta_4 Smoking_i + \beta_5 Menses_{ij} + \beta_6 Study_i \\ + \beta_7 Col_time_{ij} + \beta_8 Fever_{ij} + \beta_9 Second_Pol_{ij} + \beta_{10} Sex_i + \beta_{11} Age_i + P_i + W_{ij} + \varepsilon$$

B1.2.3 The association between air pollutant exposure and 8-OHdG and MDA controlling for aMT6s as a covariate in LMER models

LMER models were developed to investigate the relationship between pollutant exposure and 8-OHdG and MDA with adjusting for aMT6s. The dependent and independent variables were the same as described in B1.2.2. In addition to the fixed-effect covariates described above (1.1), aMT6s was added as another covariate and a covariate, the time of day, was removed due to the collinearity issue with aMT6s as following

Formula B4. The stratified analyses for the exposure-response relationships for 8-OHdG and MDA based on aMT6s level (cut point 10.3 ng/mg) were conducted following

Formula B4. The second pollutant models were shown as described in *Formula B5.* To investigate whether aMT6s modify the relationship between air pollutant exposure and biomarker of oxidative stress, the interaction term between urinary aMT6s and air pollutant exposure was added as shown in *Formula B6.*

Formula B4

$$Y_{ij} \sim \beta_0 + \beta_1 Pol_{ij} + \beta_2 RH_{ij} + \beta_3 Temp_{ij} + \beta_4 Smoking_i + \beta_5 Menses_{ij} + \beta_6 Study_i + \beta_7 Fever_{ij} + \beta_8 aMT6s_{ij} + \beta_9 Sex_i + \beta_{10} Age_i + P_i + W_{ij} + \varepsilon$$

Formula B5

$$Y_{ij} \sim \beta_0 + \beta_1 Pol_{ij} + \beta_2 RH_{ij} + \beta_3 Temp_{ij} + \beta_4 Smoking_i + \beta_5 Menses_{ij} + \beta_6 Study_i + \beta_7 Fever_{ij} + \beta_8 aMT6s_{ij} + \beta_9 Second_Pol_{ij} + \beta_{10} Sex_i + \beta_{11} Age_i + P_i + W_{ij} + \varepsilon$$

Formula B6

$$Y_{ij} \sim \beta_0 + \beta_1 Pol_{ij} + \beta_2 RH_{ij} + \beta_3 Temp_{ij} + \beta_4 Smoking_i + \beta_5 Menses_{ij} + \beta_6 Study_i + \beta_7 Fever_{ij} + \beta_8 aMT6s_{ij} + \beta_9 aMT6s_{ij} \times Pol_{ij} + \beta_{10} Sex_i + \beta_{11} Age_i + P_i + W_{ij} + \varepsilon$$

Codebook

i: the participant id number (*i*=1, 2, ..., 159)

j: the sample number (*j*=1,2,...,8)

Y_{ij}: the natural logarithm-transformed concentration of urinary biomarkers of oxidative stress (ng/mg creatinine)

Pol_{ij}: the 12h or 24h personal air pollutant exposure (PM_{2.5}, O₃, NO₂)

Second_Pol_{ij}: the 12h or 24h personal air pollutant exposure (PM_{2.5}, O₃, NO₂) controlled as the second pollutant.

RH_{ij}: the average 12h or 24h ambient relative humidity prior to sample collection (%).

Temp_{ij}: the average 12h or 24h outdoor temperature prior to sample collection (unit: °C)

Smoking_i: the current smoking status of subject (0=non-current smoker, 1=current smoker)

Menses_{ij}: the menstruation status (0=male, 1=female-non-menses, 2=female-menses)

Study_i: in which study the sample was belong to (0=Shanghai, 1=Changsha)

Fever_{ij}: the respiratory infection status (0=non-respiratory infection, 1=respiratory infection)

Col_time_{ij}: the time of sample collection (0=first morning, 1=daytime)

aMT6s_{ij}: the natural logarithm-transformed concentration of urinary aMT6s (ng/mg creatinine)

aMT6s_{ij}×Pol_{ij}: the interaction between air pollutant exposure and urinary aMT6s.

Sex_i: the sex of subject (0=male, 1=female)

Age_i: the Age of subject

P_i: the individual-specific random intercept

W_{ij}: the random intercept for the day of week that urine collected

ε: residual

B2. Sensitivity analyses

B2.1 Two-pollutant models

Two-pollutant models, following *Formula B3 and B5*, were developed to examine whether the dose-response relationship obtained in the single-pollutant models can be retained after controlling for a second co-pollutant. The dose-response relationship between 12h air pollutant exposure and aMT6s were not remarkably changed after controlling for any of two co-pollutants regarding to the significance and effect size (Figure B1).

Similarly, no noticeable changes in the dose-response relationship between 12h air pollutant exposure and 8-OHdG and MDA without adjusting for aMT6s were found after controlling for another co-pollutant. In models adjusting aMT6s as a covariate, the relationship between NO₂ and 8-OHdG were changed from non-significant to be significantly positive after controlling for O₃. However, the relationship between NO₂ and MDA and between PM_{2.5} and MDA were changed to be non-significant after controlling for PM_{2.5} and NO₂, respectively. No noticeable changes were also found in the association between aMT6s normalized 8-OHdG and MDA and 12h air pollutant exposure after adjusting for the second pollutant, except the significant positive association between MDA and 12h PM_{2.5} exposure and between MDA and 12h NO₂ became non-significant after controlling for NO₂ and PM_{2.5}, respectively (Figure B2).

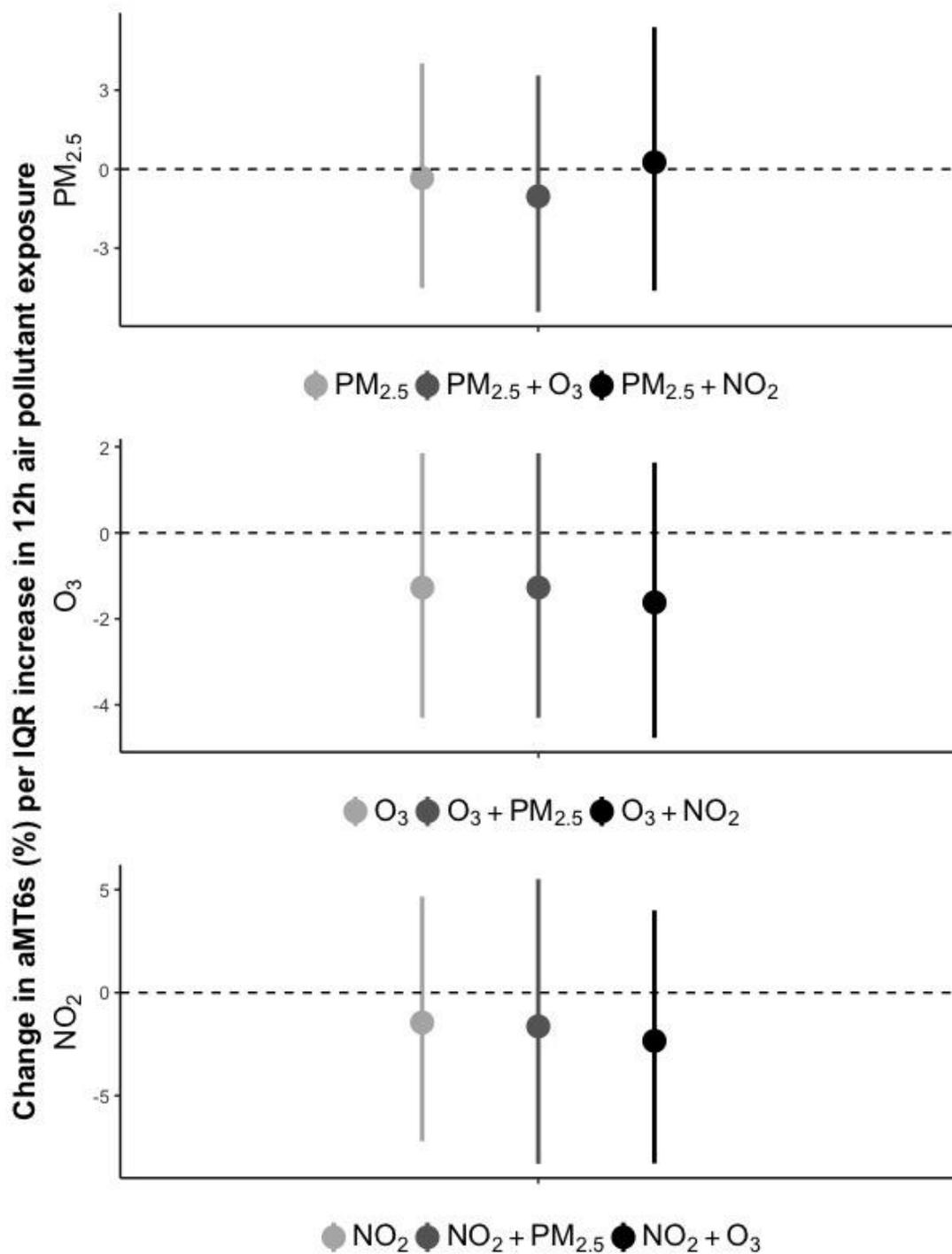


Figure B1. Estimated means and 95% confident intervals for change in aMT6s (%) per IQR increase in 12h PM_{2.5}, O₃, and NO₂ exposure from the single and two-pollutant models.

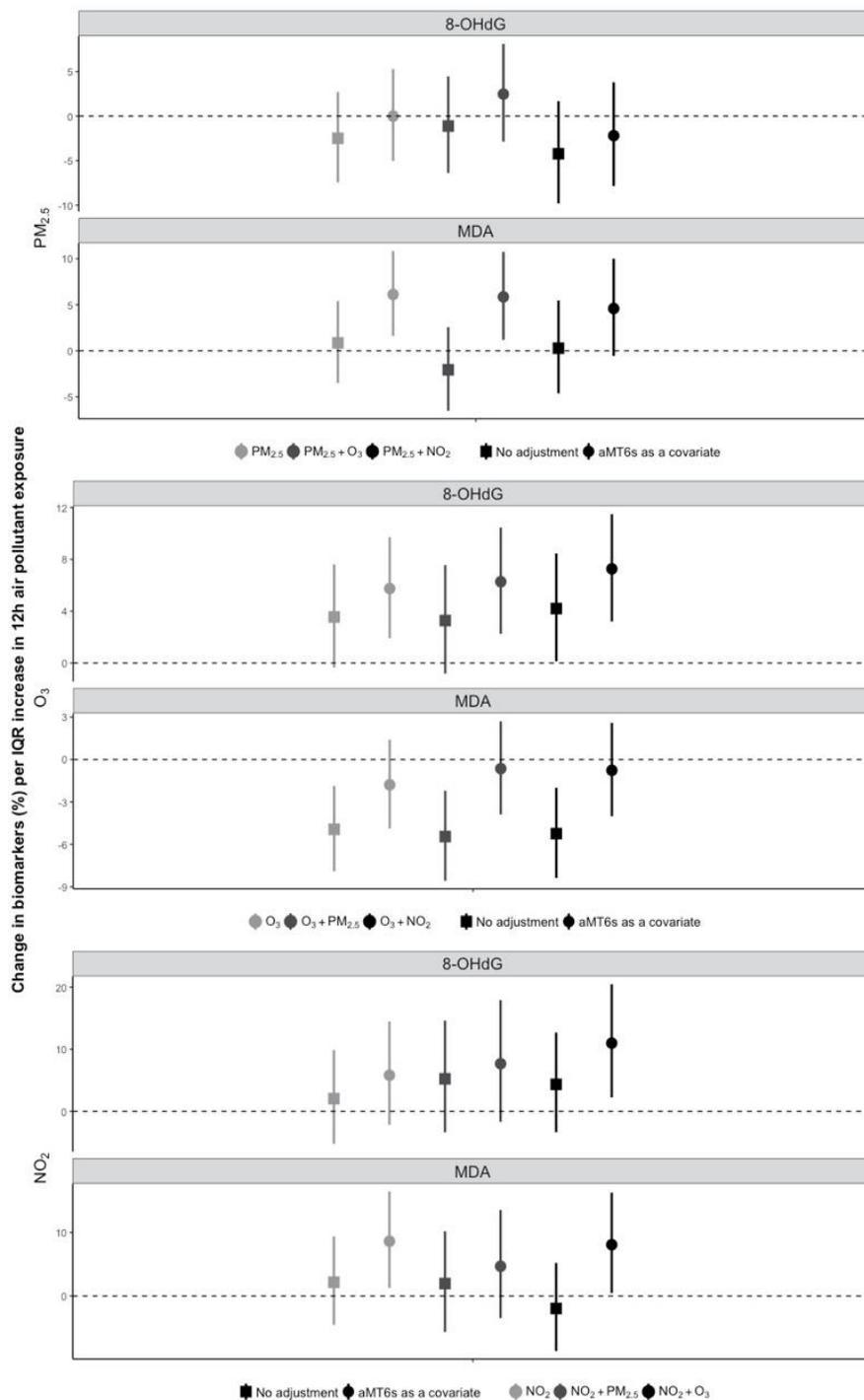


Figure B2. Estimated means and 95% confident intervals for change in biomarkers (%) with one IQR increase in 12h PM_{2.5}, O₃, and NO₂ exposure from the single and two-pollutant models.

B2.2 Excluding current smokers, subjects undergoing menstruation, and subjects reporting respiratory infection.

Sensitivity analyses were conducted for the associations shown in the main content in dataset removing measurements of current smokers and measurements of subjects undergoing menstruation and respiratory inflammation when the urine samples were collected following Formula B1, B2, B4, B6. 715 measurements were left, including 480 from the Shanghai Study and 235 from the Changsha Study. No noticeable changes were found for these associations in the new dataset (Figure B3-B6)

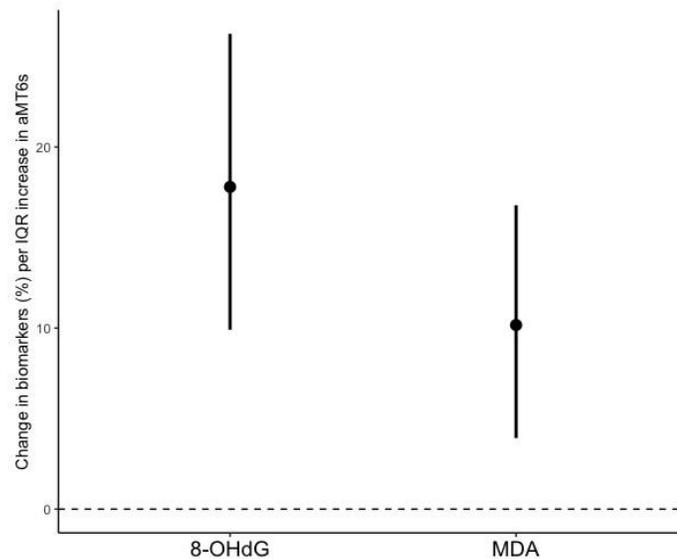


Figure B3. The percentage change in 8-OHdG and MDA per IQR increase in aMT6s (Error bar indicates the 95% confidence interval).

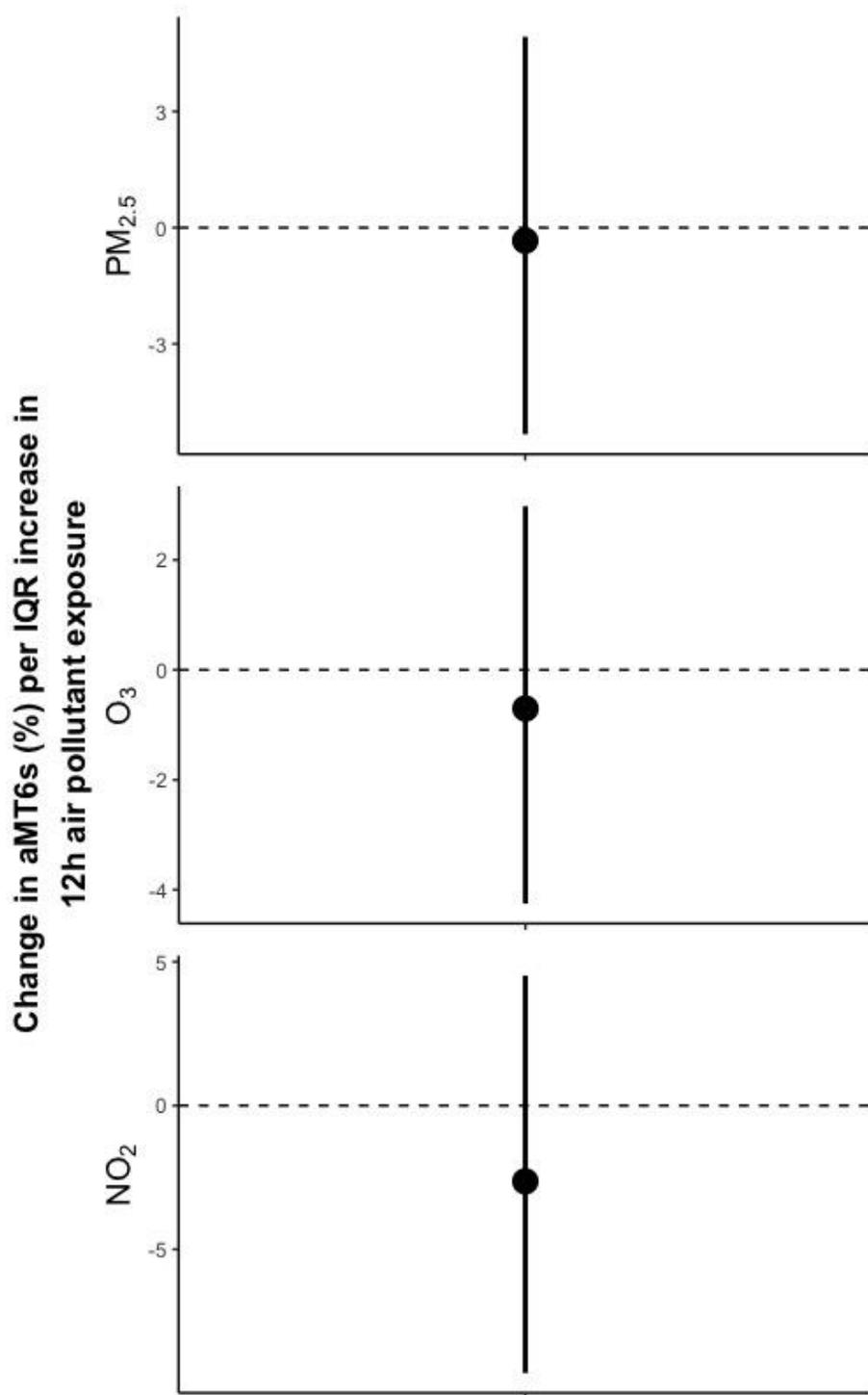


Figure B4. Estimated means and 95% confident intervals for change in aMT6s (%) per IQR increase in 12h PM_{2.5}, O₃, and NO₂ exposure from single-pollutant LMER models.

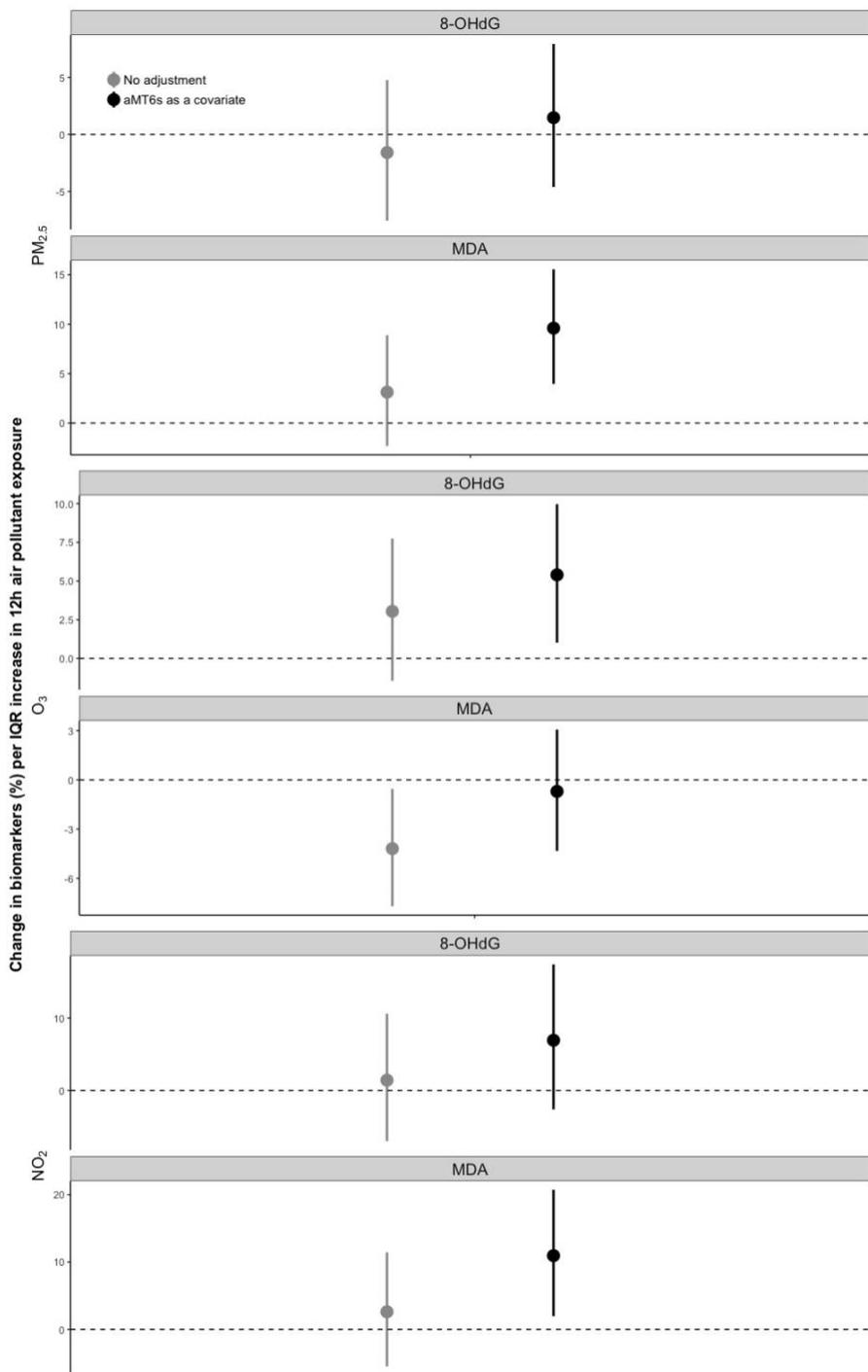


Figure B5. Estimated means and 95% confident intervals for change in biomarker (MDA, 8-OHdG) concentrations (%) with one IQR increase in 12h PM_{2.5}, O₃, and NO₂ exposure from the single-pollutant models.

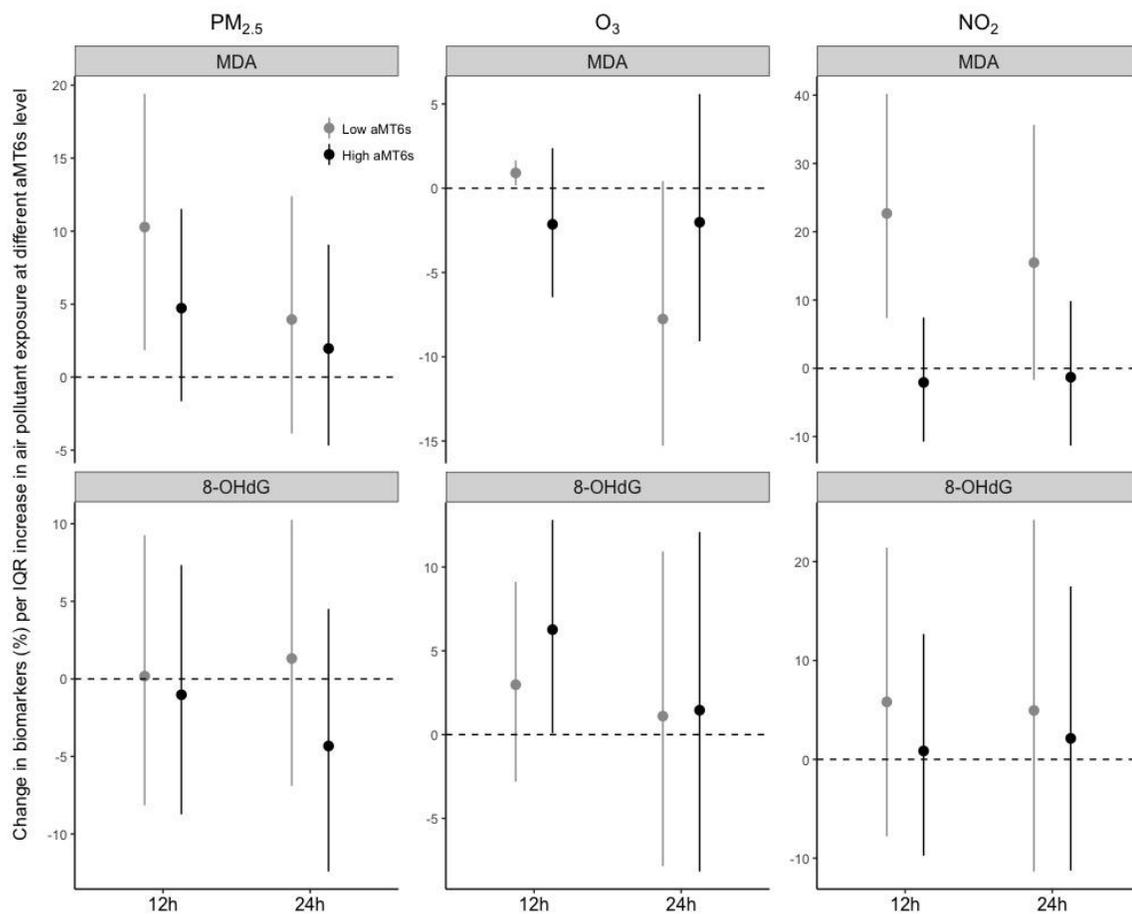


Figure B6. Estimated means and 95% confident intervals for change in biomarkers (%) with one IQR increase in PM_{2.5}, O₃, and NO₂ from the single-pollutant models. Low aMT6s: ≤10.2 ng/mg; High aMT6s: >10.2 ng/mg.

B2.3 Separate analyses for the Shanghai and the Changsha Studies

We conducted separate analyses for the Shanghai and Changsha studies, respectively, on all the associations analyzed using the combined dataset following Formula B1, B2, B4, B6. No remarkable changes were found for the association of aMT6s with 8-OHdG or MDA (see Figure B7) and the association between 12h air pollutant exposure and aMT6s (Figure B8). The associations of 12h air pollutant exposure with 8-OHdG or MDA were not noticeably changed in the Shanghai Study. However, the results shown in the main content were not seen in the Changsha Study, except the relationship between 12h O₃ and 8-OHdG (Figure B9).

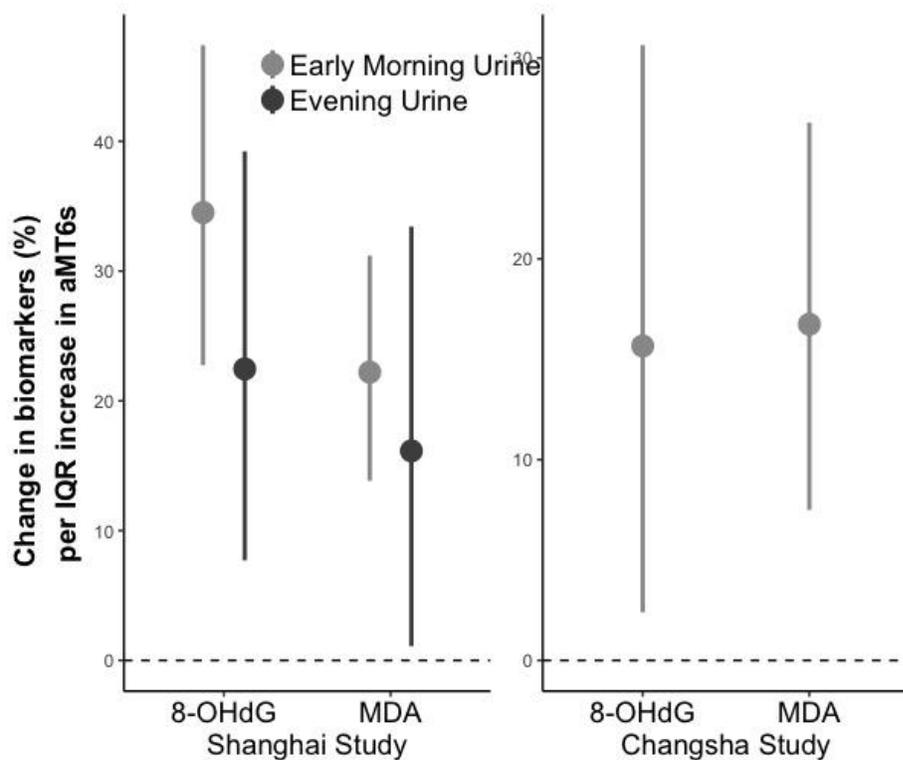


Figure B7. The percentage change in 8-OHdG and MDA per IQR increase in aMT6s in the Shanghai and the Changsha Study

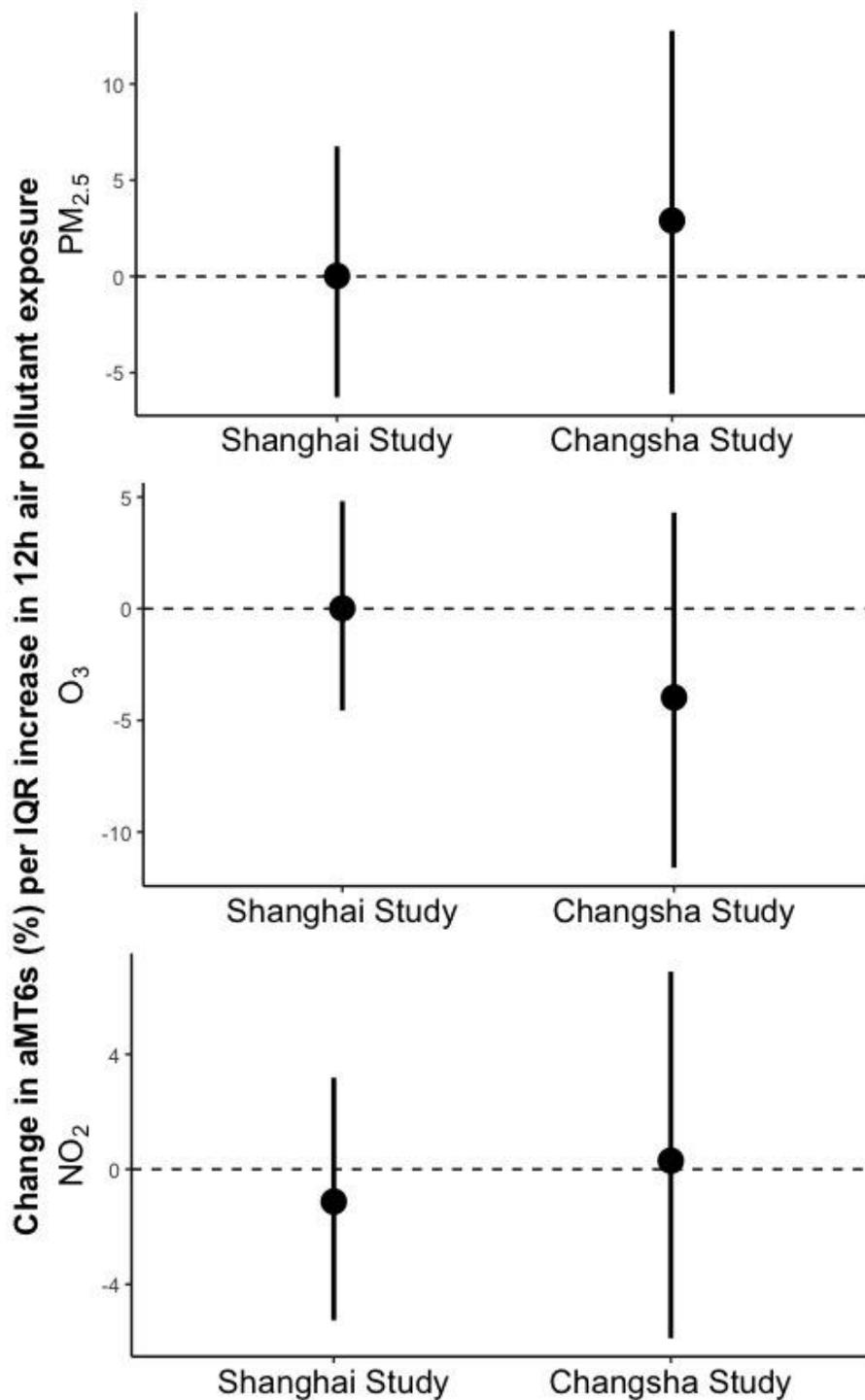


Figure B8. Estimated means and 95% confident intervals for change in aMT6s (%) per IQR increase in 12h PM_{2.5}, O₃, and NO₂ exposure in the Shanghai study and the Changsha Study from the single-pollutant models.

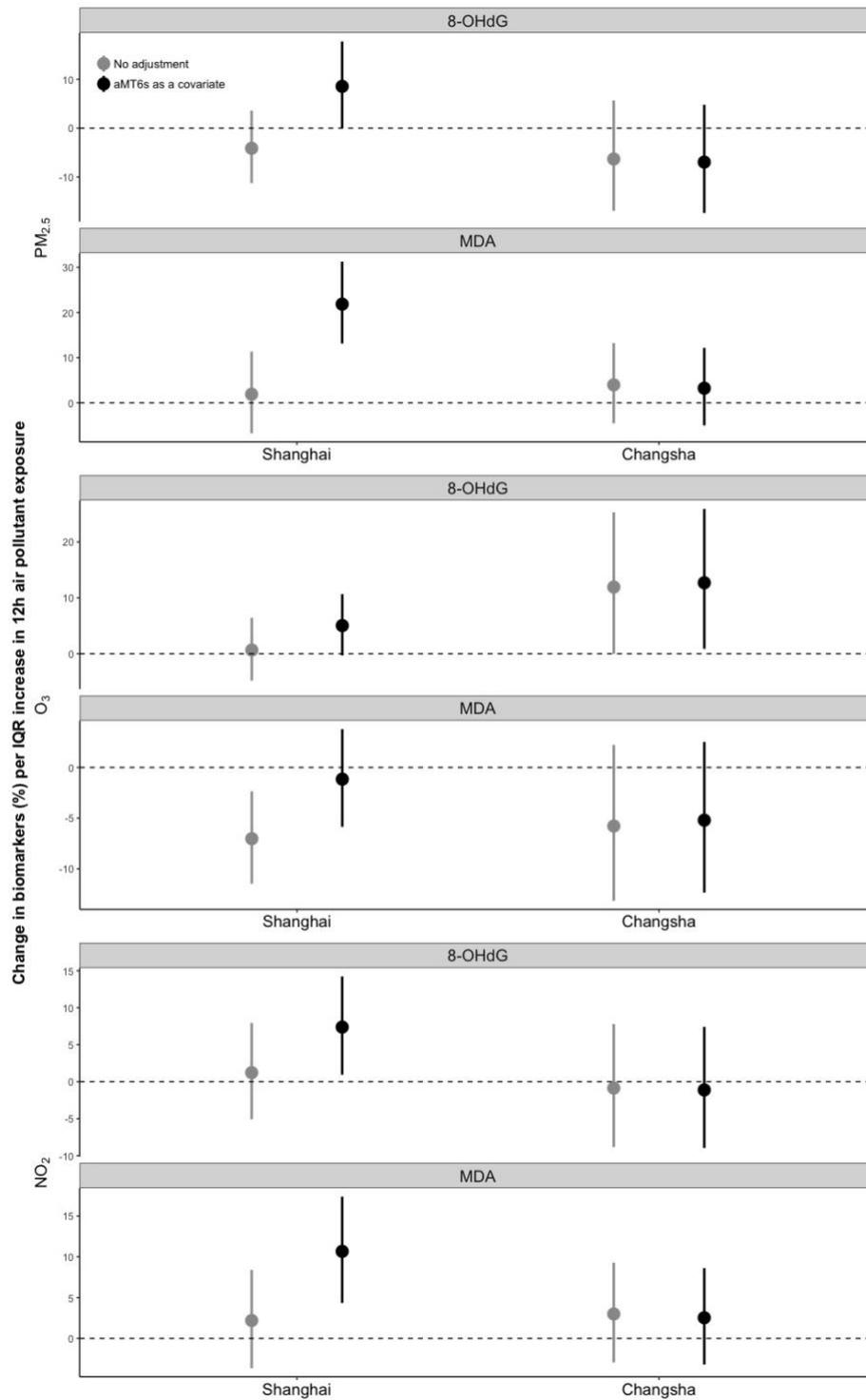


Figure B9. Estimated means and 95% confident intervals for change in biomarkers (%) with one IQR increase in 12h PM_{2.5}, O₃, and NO₂ exposure in the Shanghai Study and the Changsha Study from the single-pollutant models.

B3. Effects Modification

To investigate whether aMT6s modifies this relationship, we assessed the interaction between air pollution and aMT6s in LMER models following Formula B6. The interaction term in the models were not significant, except for the association between 24h O₃ exposure and MDA (Table B1). Therefore, within the aMT6s range (0.3-93.5 ng/mg) measured in the present study, we have insufficient evidence to support a modification effect of aMT6s.

Table B1. The interaction between 12h & 24h air pollutant exposure and aMT6s in pollutant models.

	PM _{2.5} × aMT6s interaction			O ₃ × aMT6s interaction			NO ₂ × aMT6s interaction		
	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value
12h air pollutant exposure									
8-OHdG	0.000	(-0.002, 0.002)	0.999	0.008	(-0.007, 0.023)	0.310	0.001	(-0.006, 0.007)	0.901
MDA	-0.001	(-0.003, 0.001)	0.391	0.011	(-0.002, 0.024)	0.102	0.006	(0.000, 0.011)	0.050
24h air pollutant exposure									
8-OHdG	-0.000	(-0.003, 0.002)	0.828	0.007	(-0.007, 0.020)	0.334	0.002	(-0.006, 0.009)	0.691
MDA	-0.001	(-0.003, 0.001)	0.483	0.023	(0.011, 0.034)	0.0001	0.006	(0.000, 0.013)	0.053

Appendix C

Appendix C contains the supporting information for Chapter 4, published online from Hu, X.; He, L.; Zhang, J.; Qiu, X.; Zhang, Y.; Mo, J.; Day, D. B.; Xiang, J.; Gong, J., Inflammatory and oxidative stress responses of healthy adults to changes in personal air pollutant exposure. *Environmental Pollution*. 2020, 263 (Publisher: Elsevier). X. Hu and L. He are co-first authors on the publication. Specifically, L. He contributed to research idea conception, statistical analyses, table and figure design, and writing and editing of the manuscript.

C1. Time-activity questionnaire

The questionnaire asked about time-activity over the past 7 days and during the past 24 hours. The total times in hours that subjects spent in their office, dormitories, and other locations during the past 7 days were asked. The specification as well as how many hours were spent in each of these other locations were recorded. Similarly, the questionnaire also asked how long did the subjects spent their time in their offices, dormitories, inside in other environments (and where those locations were), outside in other environments (and where those locations were) during the past 24 hours. The number of hours spent in the same room as someone smoking during the past 24 hours and the current smoking status during the past 1-week was recorded. The same data was used to extrapolate the time-activity pattern during the 7-14 days prior to each of the visits as well.

C2. Statistical analysis

C2.1 The relationships of personal air pollutant exposure and cytokines concentration.

We constructed linear mixed-effects regression (LMER) models to determine the exposure-response association between air pollutant exposure and cytokines following *Formula C1* using the *lme4* and *lmeTest* packages of the R software (version 3.6.1). All of the cytokines were natural logarithm-transformed to meet the assumptions of linear mixed-effects regression. The dependent variable in the model is each of the cytokines, and the independent variable is 12-hour, 24-hour, 1-week, or 2-week average personal air pollutant exposure before blood sample collection. Fixed-effects were also adjusted in the models including age, sex, smoking status, batch factors of cytokines measurement, respiratory infection status, and ambient temperature and relative humidity averaged over the same timeframe of personal air pollutants exposure. An individual-specific random intercept was added to account for correlation within the same participant, including sex and age. The two-pollutant models were developed following *Formula C2*.

Formula C1

$$Y_{ij} \sim \beta_0 + \beta_1 Pol_{ij} + \beta_2 RH_{ij} + \beta_3 Temp_{ij} + \beta_4 Smoking_i + \beta_5 Batch_i + \beta_6 Fever_{ij} + \beta_7 Sex_i + \beta_8 Age_i + \beta_9 SHS_{ij} + P_i + \epsilon$$

Formula C2

$$Y_{ij} \sim \beta_0 + \beta_1 Pol_{ij} + \beta_2 RH_{ij} + \beta_3 Temp_{ij} + \beta_4 Smoking_i + \beta_5 Batch_i + \beta_6 Fever_{ij} + \beta_7 Sex_i \\ + \beta_8 Age_i + \beta_9 SHS_{ij} + \beta_{10} Second_Pol_{ij} + P_i + \epsilon$$

C2.2 The effects of removing electrostatic precipitator (ESP) on cytokines concentration.

LMER models were constructed to determine the effects of removing ESP on cytokines concentration following *Formula C3*. All the concentrations of cytokines were natural logarithm-transformed before the test. The dependent variable in the model is the concentration of each of cytokines. The independent variable is the factor of with or without having ESP operated. In addition to the fixed-effect covariates described above, the ambient ozone concentration when the subject was in unfiltered environment averaged over 2-week before each of blood collections was added as another covariate to control for the natural variation in ambient ozone concentration. In these models, 2-week average ambient relative humidity and temperature were uniformly controlled to reflect the study design that blood samples were collected 2-week after removing ESP.

Formula C3

$$Y_{ij} \sim \beta_0 + \beta_1 ESP_{ij} + \beta_2 RH_2w_{ij} + \beta_3 Temp_2w_{ij} + \beta_4 Smoking_i + \beta_5 Batch_i + \beta_6 Fever_{ij} \\ + \beta_7 Amb_Ozone_Unfil_{ij} + \beta_8 Sex_i + \beta_9 Age_i + \beta_{10} SHS_{ij} + P_i + \epsilon$$

C2.3 Power analysis

Before conducting this study, we used priori test using MANOVA model to determine the statistical power of our analysis. In this test, we used the following parameter. The effect size is 0.15, which is a conservative estimation of the effective size of 2-week ozone exposure on the inflammatory cytokines. The α is 0.05, number of measurements is 3, and the correlation among repeated measurement is assumed as 0.7. A sample size of 53 (145 total measurements) would obtain a statistical power of 0.99.

We also conducted a post-hoc power test using the effect sizes obtained from our model results. The average effect size of the 2-week O₃ exposure on the inflammatory cytokines is 0.133. Using the other parameter described above, we could obtain a post-hoc statistical power of 0.99.

Codebook

i : the participant id number ($i= 1, 2, \dots, 53$)

j : the sample number ($j= 1, 2, 3$)

Y_{ij} : the natural logarithm-transformed concentration of each of cytokines (pg/mL)

Pol_{ij} : the 12h, 24h, 1-week, or 2-week personal air pollutant exposure (PM_{2.5}, O₃, NO₂, SO₂)

RH_{ij} : the average 12h, 24h, 1-week, or 2-week ambient relative humidity before blood collection (%).

$Temp_{ij}$: the average 12h, 24h, 1-week, or 2-week ambient temperature before sample collection (unit: °C)

$Smoking_i$: the current smoking status of the subject (0=non-current smoker, 1=current smoker)

$Batch_k$: in which batch the sample was analyzed ($k=1, 2$)

$Fever_{ij}$: the respiratory infection status (0=non-respiratory infection, 1=respiratory infection)

$Amb_Ozone_Unfil_{ij}$: the 2-week average ambient ozone concentration when the subject was in the unfiltered environment (ppb)

RH_2w_{ij} : the average 2-week ambient relative humidity before blood collection (%).

$Temp_2w_{ij}$: the average 2-week ambient temperature before sample collection (unit: °C)

Sex_i : the sex of subject (0=male, 1=female)

Age_i : the age of the subject

SHS_{ij} : the number of hours spent in the same room as someone smoking during the past 24 hours

P_i : the individual-specific random intercept

\mathcal{E} : residual

C3. Pearson correlation among ambient pollutants

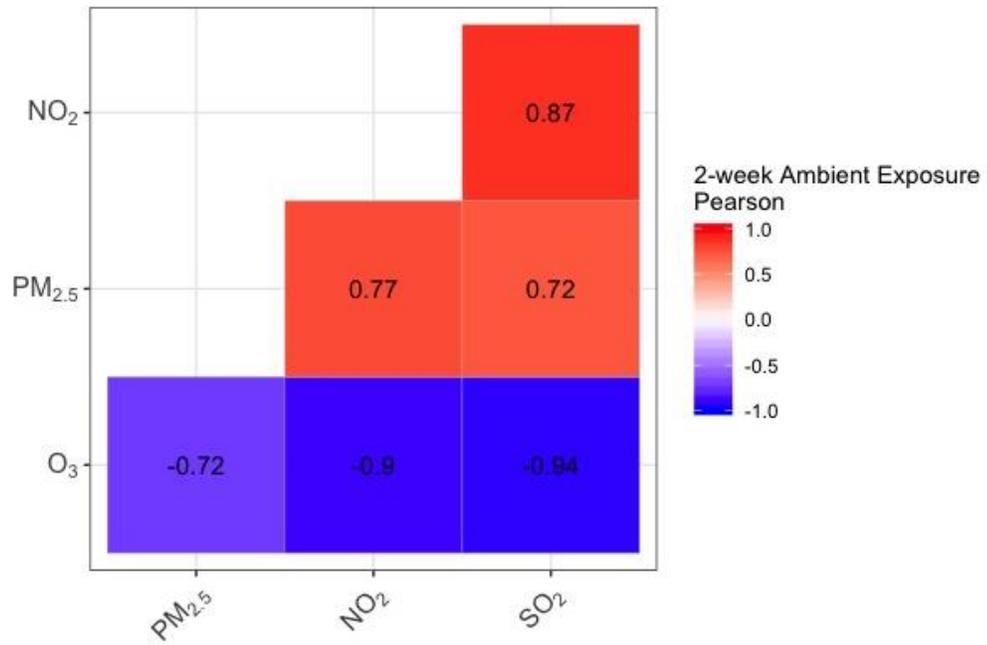


Figure.C1. Pearson correlation coefficients among 2-week average ambient air pollutants concentrations.

C4. Detailed model results

Table C1 Detailed single-pollutant models results (i.e. effect size, CIs, and p-value) and adjusted p-value (For Figure 10).

Pollutant Biomarkers	Time	O ₃				PM _{2.5}				NO ₂				SO ₂			
		95 % CIs	Effec	p.value	Adjusted	95 % CIs	Effec	p.value	Adjuste	95 % CIs	Effec	p.value	Adjuste	95 % CIs	Effec	p.value	Adjusted
IL1β	12h	-13.8 to 8.75	-3.22	0.58	0.64	-18.9 to 4.44	-7.97	0.20	0.39	8.21 to 36.5	21.6	0.0012	0.0024	-2.03 to 24.4	10.4	0.10	0.21
	24h	-12.2 to 10	-1.72	0.76	0.85	-15.6 to 7.4	-4.78	0.42	0.61	-2.55 to 22.9	9.45	0.13	0.18	-9.98 to 12.5	-0.65	0.91	0.91
	1w	7.2 to 54.9	28.9	0.0075	0.019	-27.4 to -7.08	-17.9	0.0020	0.0068	-26.9 to -6.1	-17.1	0.0036	0.0089	-32.2 to -5.89	-20.1	0.0077	0.015
	2w	15.7 to 69.8	40.2	0.00069	0.0017	-33.6 to -9.6	-22.5	0.0014	0.0074	-27.2 to 1.48	-14.1	0.074	0.11	-38 to -7.73	-24.3	0.0063	0.016
IL2	12h	-11.6 to 6.59	-2.92	0.53	0.64	-16 to 3.54	-6.76	0.19	0.39	6.0 to 28.1	16.5	0.0018	0.0031	-0.45 to 20.9	9.71	0.061	0.21
	24h	-11.3 to 6.25	-2.92	0.52	0.74	-13.2 to 5.7	-4.2	0.39	0.61	-1.46 to 19.1	8.3	0.097	0.17	-6.46 to 12.2	2.45	0.60	0.73
	1w	6.78 to 43.6	23.8	0.0052	0.017	-24 to -7.02	-15.9	0.00090	0.0045	-23.1 to -5.7	-14.9	0.0023	0.0078	-27.9 to -5.7	-17.5	0.0054	0.013
	2w	12.2 to 53.8	31.4	0.00087	0.0017	-28.6 to -7.89	-18.9	0.0015	0.0074	-24.1 to -0.56	-13.1	0.041	0.084	-31.8 to -5.5	-19.7	0.0088	0.018
IL4	12h	-49.5 to 10.4	-25.3	0.14	0.28	-4.93 to 109	40.9	0.087	0.39	-19.8 to 79.9	20.1	0.37	0.37	-27 to 59.5	7.93	0.70	0.70
	24h	-55.5 to -3.47	-34.4	0.033	0.066	-7.4 to 104	37.4	0.11	0.44	-6.37 to 106	39	0.10	0.17	-1.19 to 107	43	0.058	0.20
	1w	-25.9 to 137	32.6	0.34	0.42	-41.6 to 26.9	-13.9	0.45	0.45	-45.2 to 19.4	-19.1	0.28	0.31	-52.5 to 31.1	-21.1	0.36	0.40
	2w	-4.93 to 220	74.3	0.072	0.080	-47 to 41.1	-13.5	0.56	0.56	-66.3 to -9.2	-44.7	0.020	0.066	-67.4 to 13.8	-39.1	0.12	0.12
IL6	12h	-24.9 to -5.9	-15.9	0.0029	0.0073	-9.02 to 17.5	3.39	0.61	0.77	12.9 to 42.4	26.8	9.37E-05	0.00047	-1.46 to 27	11.9	0.083	0.21
	24h	-24.5 to -6.67	-16.1	0.0015	0.0050	-6.87 to 18.3	4.97	0.42	0.61	11 to 38.8	24.1	0.00023	0.0011	-0.49 to 24.8	11.5	0.061	0.20
	1w	1.66 to 45.6	21.7	0.033	0.066	-25.9 to -5.99	-16.6	0.0033	0.0082	-25.1 to -4.7	-15.5	0.0067	0.013	-32.1 to -7.07	-20.6	0.0044	0.013
	2w	18.7 to 70.8	42.4	0.00021	0.0013	-30.4 to -5.53	-18.9	0.0076	0.017	-32.2 to -7.8	-20.9	0.0030	0.0030	-40.9 to -13.9	-28.7	0.00055	0.0028
IL8	12h	-9.93 to 11.1	0.029	1.0	1.0	-15.9 to 7.11	-5.1	0.39	0.66	2.54 to 28.2	14.6	0.017	0.024	-0.75 to 24.9	11.4	0.067	0.21
	24h	-8.8 to 11.3	0.73	0.88	0.88	-14.7 to 6.03	-4.9	0.36	0.61	-5.9 to 16.8	4.8	0.39	0.43	-6.17 to 15.1	3.9	0.46	0.73
	1w	10.5 to 50.8	29.1	0.0016	0.0079	-25.8 to -7.96	-17.4	0.00069	0.0045	-25.9 to -8.24	-17.5	0.00054	0.0046	-32.8 to -10.6	-22.5	0.00060	0.0060
	2w	15.7 to 60.7	36.4	0.00031	0.0013	-26.3 to -1.8	-14.9	0.028	0.039	-27.2 to -4.03	-16.4	0.012	0.058	-38 to -13.1	-26.6	0.00046	0.0028
IL10	12h	-43.8 to -12.3	-29.8	0.0021	0.0070	-25.5 to 24.6	-3.65	0.78	0.86	30.8 to 108	64.9	4.2E-05	0.00042	-9.02 to 51.5	17.4	0.22	0.31
	24h	-41.4 to -8.69	-26.8	0.0062	0.016	-24.2 to 23.6	-3.17	0.79	0.79	21.7 to 94.1	53.7	4.04E-04	0.0013	-7.05 to 48.6	17.5	0.18	0.42
	1w	-22.3 to 57.3	-10.5	0.57	0.59	-31.6 to 10	-13.2	0.24	0.30	-32.8 to 8.24	-14.7	0.19	0.24	-40.6 to 11	-18.8	0.19	0.27
	2w	-5.27 to 101	37.9	0.093	0.093	-41.9 to 6.3	-21.4	0.12	0.13	-34.4 to 21.4	-10.8	0.46	0.46	-53.5 to -0.83	-32.1	0.045	0.050
IL17A	12h	-23.1 to -8.06	-15.9	0.00022	0.0011	-3.07 to 20.5	8.08	0.16	0.39	7.87 to 31	18.9	0.00064	0.0016	0.37 to 23.7	11.4	0.043	0.21
	24h	-23.4 to -9.17	-16.6	6.05E-05	0.00061	-2.36 to 19.9	8.19	0.13	0.44	9.64 to 32.3	20.4	0.00016	0.0011	1.22 to 22.3	11.3	0.027	0.20
	1w	-2.76 to 30	12.4	0.11	0.16	-18 to -0.12	-9.5	0.047	0.068	-15.5 to 3.45	-6.5	0.19	0.24	-19.6 to 5.09	-8.1	0.21	0.27
	2w	13.8 to 53.7	32.3	0.00038	0.0013	-25 to -3.95	-15.1	0.0099	0.017	-22.5 to 0.30	-11.8	0.055	0.092	-27.9 to -1.13	-15.6	0.036	0.045
IFN-γ	12h	-29.5 to -10.9	-20.7	0.00017	0.0011	-14.4 to 14.9	-0.85	0.91	0.91	10.8 to 43.1	25.9	0.00055	0.0016	-3.17 to 28.5	11.6	0.13	0.21
	24h	-28.4 to -9.96	-19.7	0.00026	0.0013	-10.7 to 17.8	2.58	0.72	0.79	8.52 to 39.6	23.1	0.0015	0.0037	-4.52 to 23.3	8.49	0.21	0.42
	1w	-13.5 to 28.8	5.55	0.59	0.59	-18.3 to 7.12	-6.45	0.33	0.37	-16.2 to 10.2	-3.88	0.57	0.57	-20.9 to 13.6	-5.2	0.56	0.56
	2w	15.1 to 72.8	41.1	0.0011	0.0019	-32.5 to -5.49	-20.1	0.0094	0.017	-22.5 to 10.1	-7.6	0.37	0.41	-36.7 to -3.18	-21.7	0.024	0.035
TNF-α	12h	-4.57 to 15.7	5.06	0.31	0.52	-11.9 to 7.83	-2.53	0.62	0.77	-2.82 to 18.7	7.4	0.16	0.18	-5.82 to 14.7	3.95	0.44	0.55
	24h	-4.2 to 15.7	5.29	0.28	0.46	-11.7 to 7.57	-2.53	0.61	0.76	-9.99 to 9.39	-0.77	0.88	0.88	-11 to 6.99	-2.43	0.60	0.73
	1w	10.5 to 48.2	27.9	0.0012	0.0080	-21 to -3.46	-12.7	0.0085	0.017	-23.5 to -6.82	-15.6	0.00092	0.0046	-28 to -6.61	-18	0.0031	0.013
	2w	6.88 to 45.7	24.8	0.0055	0.0068	-21.2 to 1.67	-10.5	0.087	0.11	-23.1 to -0.50	-12.5	0.042	0.084	-31.5 to -6.11	-19.8	0.0065	0.016
MDA	12h	-13.8 to 4.2	-5.25	0.26	0.26	-2.43 to 19.1	7.8	0.14	0.14	-6.45 to 13.9	3.24	0.52	0.52	-2.86 to 18.2	7.18	0.17	0.17
	24h	-14.7 to 3.9	-5.86	0.23	0.23	-5.09 to 16.8	5.27	0.33	0.33	-4.41 to 16.7	5.62	0.28	0.28	-2.25 to 18.5	7.62	0.13	0.13
	1w	-22.4 to 7.1	-8.84	0.26	0.26	-5.76 to 16.2	4.6	0.39	0.39	-4.42 to 18.1	6.27	0.26	0.26	-5.45 to 24.3	8.4	0.25	0.25
	2w	-23.4 to 7.29	-9.35	0.25	0.25	-11.4 to 15	0.90	0.89	0.89	-11 to 17.4	2.24	0.75	0.75	-8.2 to 28.4	8.56	0.33	0.33

Table C2 Detailed intervention effect model results (i.e. effect size, CIs, and p-value) and adjusted p-value (For Figure 12).

Biomarker	Effect size	95 % CIs	p value	Adjusted p value
IL1 β	-64.7	-85 to -16.8	0.018	0.037
IL2	-57.5	-79.1 to -13.6	0.019	0.037
IL6	-72.4	-86.8 to -42.2	0.00085	0.0042
IL8	-73.6	-86.7 to -47.7	0.00023	0.0023
IL17A	-51.4	-74.7 to -6.61	0.031	0.044
IFN- γ	-55.6	-81.7 to 8.01	0.073	0.083
TNF- α	-61.4	-80.1 to -25.5	0.0051	0.017
IL4	-90	-99.2 to 27.1	0.075	0.084
IL10	-72.3	-94.2 to 31.8	0.11	0.11
MDA	10.6	-42.2 to 115	0.76	0.76

C5. Ambient air pollution level

Table C3 Ambient pollutant exposure averaged over 12-hour, 24-hour, 1-week and 2-week before biospecimen collections.

	Before ESP Removal (Visit 1), Mean \pm SD	After ESP Removal (Visit 2), Mean \pm SD	After ESP Removal (Visit 3), Mean \pm SD
12-hour Average Ambient Pollutant			
O ₃ (ppb)	39.5 \pm 2.35	13.3 \pm 3.83	16.8 \pm 6.89
PM _{2.5} (μ g/m ³)	44.4 \pm 9.5	122 \pm 54.8	136 \pm 14.5
NO ₂ (ppb)	28.7 \pm 3.1	40.7 \pm 7.23	27.1 \pm 4.6
SO ₂ (ppb)	10.7 \pm 1.6	13.7 \pm 3.1	11.4 \pm 3.28
24-hour Average Ambient Pollutant			
O ₃ (ppb)	43.4 \pm 3.54	18.1 \pm 3.71	16.0 \pm 5.32
PM _{2.5} (μ g/m ³)	44.9 \pm 9.96	115 \pm 57.9	142 \pm 23.6
NO ₂ (ppb)	26.3 \pm 4.2	37.3 \pm 7.21	32.8 \pm 3.39
SO ₂ (ppb)	10.5 \pm 1.6	14.6 \pm 1.90	13.2 \pm 3.89
1-week Average Ambient Pollutant			
O ₃ (ppb)	38.6 \pm 0.54	19.2 \pm 0.62	20.6 \pm 1.63
PM _{2.5} (μ g/m ³)	39.3 \pm 0.46	69.4 \pm 21.3	107 \pm 21.5
NO ₂ (ppb)	22.1 \pm 1.17	30.7 \pm 2.66	37.3 \pm 3.67
SO ₂ (ppb)	9.19 \pm 0.29	11.0 \pm 0.91	13.7 \pm 0.86
2-week Average Ambient Pollutant			
O ₃ (ppb)	31.4 \pm 1.86	25.5 \pm 2.66	20.4 \pm 0.37
PM _{2.5} (μ g/m ³)	72.8 \pm 2.73	63.2 \pm 8.41	107.8 \pm 3.75
NO ₂ (ppb)	28.4 \pm 1.62	31.0 \pm 1.25	35.7 \pm 2.36
SO ₂ (ppb)	8.3 \pm 0.071	10.8 \pm 0.38	13.2 \pm 0.14

C6. Two-pollutant model results for NO₂, PM_{2.5}, and SO₂

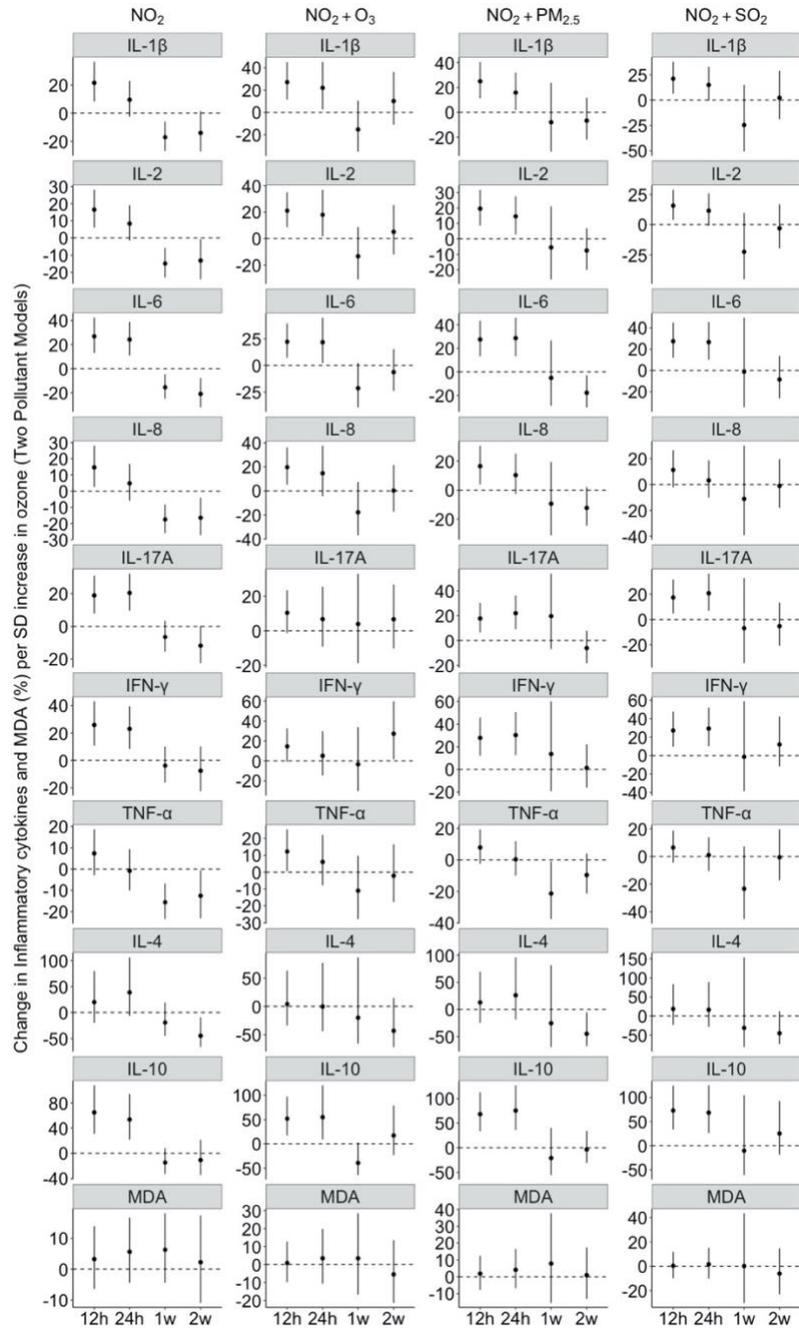


Figure.C2. Mean change in biomarkers (%) and 95% CIs associated with one SD increase in 12-hour, 24-hour, 1-week and 2-week averaged NO₂ exposure from two-pollutant models.

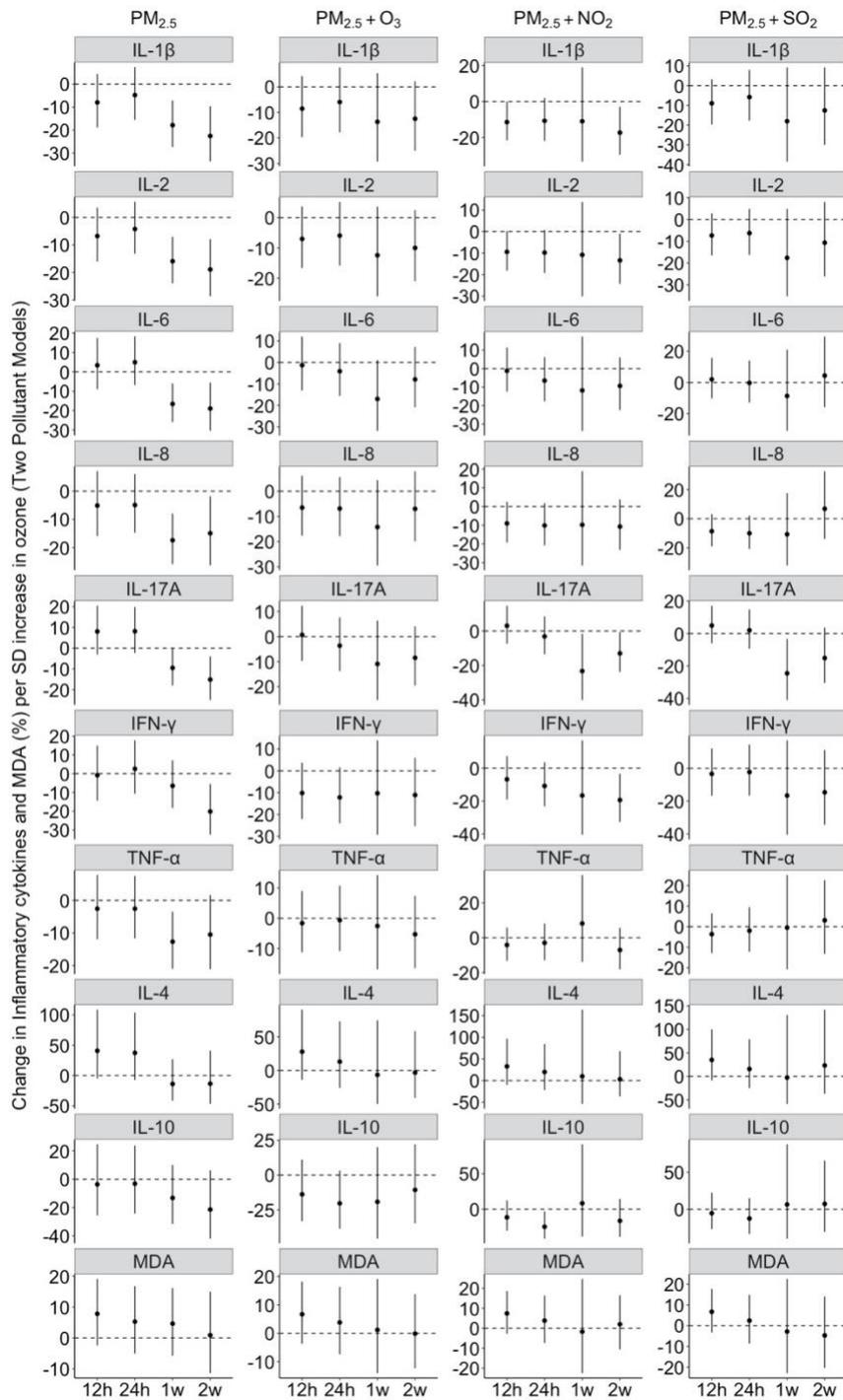


Figure.C3. Mean change in biomarkers (%) and 95% CIs associated with one SD increase in 12-hour, 24-hour, 1-week and 2-week averaged PM_{2.5} exposure from two-pollutant models.

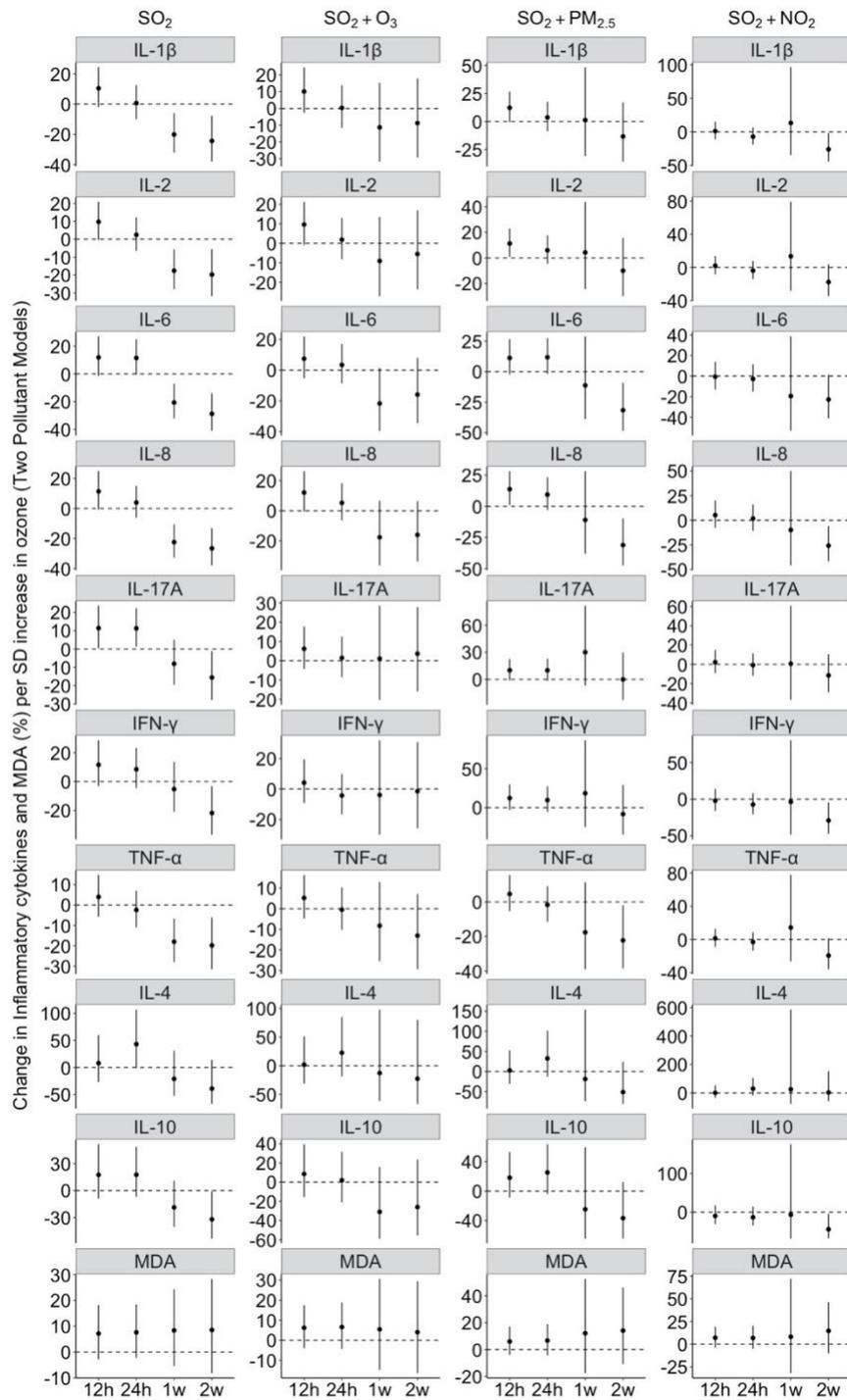


Figure.C4. Mean change in biomarkers (%) and 95% CIs associated with one SD increase in 12-hour, 24-hour, 1-week and 2-week averaged SO₂ exposure from two-pollutant models.

C7. Sensitivity analyses

C7.1 Excluding subjects undergoing respiratory infection and current smokers.

Sensitivity analyses were conducted for the exposure-response associations shown in the main content in dataset removing either (1) measurements of subjects undergoing respiratory infection during one week before the blood samples collection or (2) measurements from current smokers following Formula C1 and C2. For (1), after removing measurements of subjects undergoing respiratory infection, 132 measurements were included (13 were removed). For (2), after removing current smokers, a dataset of 118 measurements were included (27 were removed).

As shown in Figure.C5, compared with the main content, no noticeable changes were found for the relationships of O₃ exposure and cytokines concentrations after excluding the measurements undergoing respiratory infection. Similarly, after excluding measurement of current smokers, we found that the associations of personal O₃ exposure with cytokines were not remarkably changed from the results shown in the main text, in terms of effect sizes and statistical significance (Figure.C6).

In sum, by comparing the effect sizes of association shown in the sensitivity analyses and main analyses, we concluded that sensitivity analyses

did not change the conclusion made in the main analyses and supported the robustness of the conclusion.

(1) Excluding subjects undergoing respiratory infection.

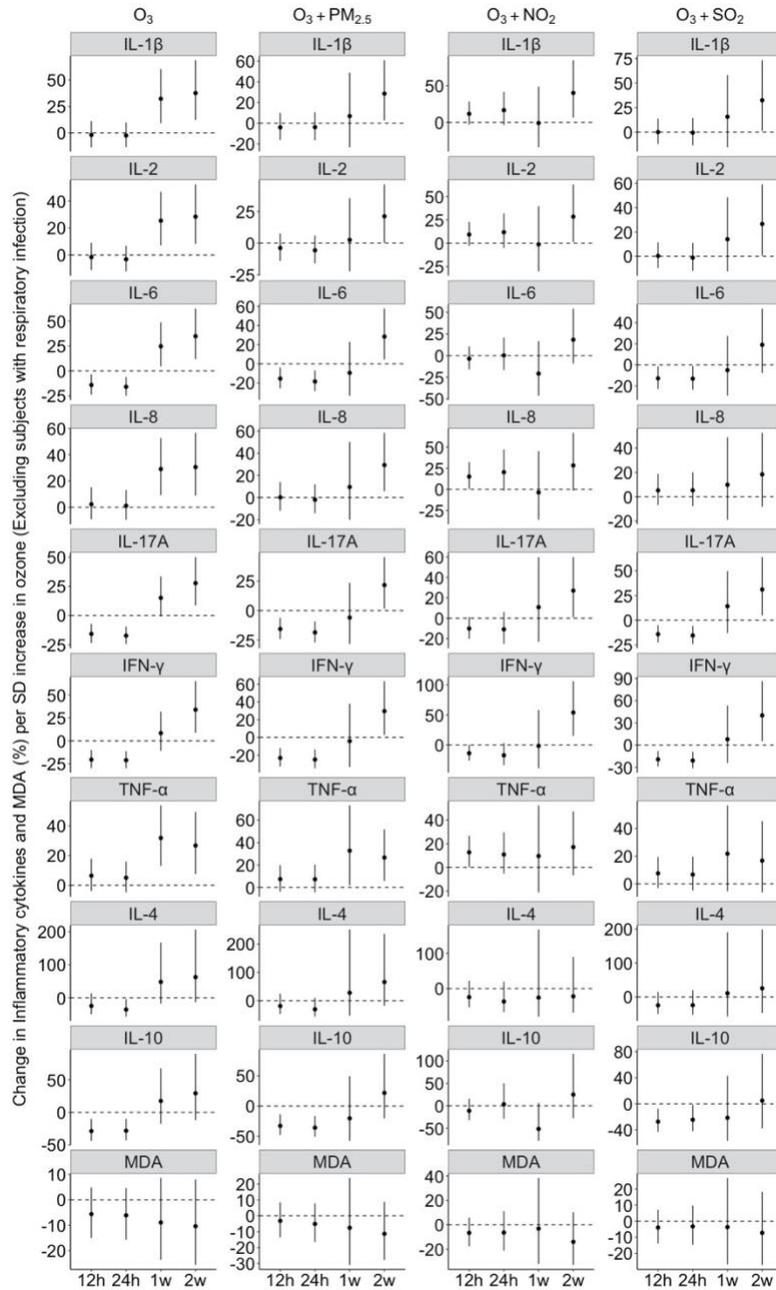


Figure.C5. Mean change in biomarkers (%) and 95% CIs associated with one SD increase in 12-hour, 24-hour, 1-week and 2-week averaged O₃ exposure: single- and two-pollutant models (Excluding subjects undergoing respiratory infection)

(2) Excluding current smokers.

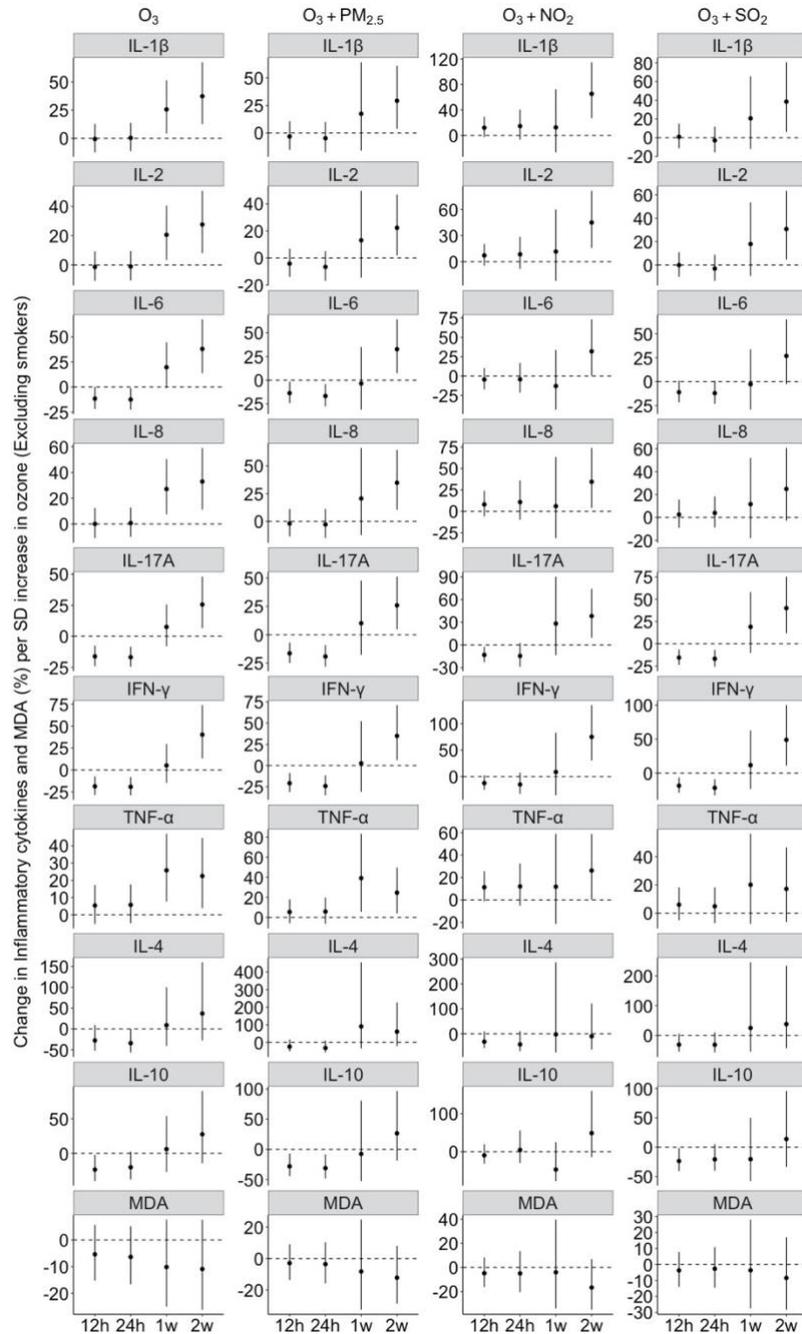


Figure.C6. Mean change in biomarkers (%) and 95% CIs associated with one SD increase in 12-hour, 24-hour, 1-week and 2-week averaged O_3 exposure: single- and two-pollutant models (Excluding current smokers).

Appendix D

Appendix D contains the supporting information for Chapter 5, published online from He, L.; Hu, X.; Gong, J., Day, D. B.; Xiang, J.; Mo, J.; Zhang, Y.; Zhang, J.; Endogenous melatonin mediation of systemic inflammatory responses to ozone exposure in healthy adults. *Science of The Total Environment*. 2020 (Publisher: Elsevier).

D1. Statistical analysis

D1.1 Association between air pollutant exposure and urinary aMT6s

We used linear mixed-effects regression (LMER) models to determine the association between air pollutant exposure and aMT6s following Formula D1 using the lme4 and lmeTest packages of the R software (version 3.6.1). The aMT6s concentration and personal air pollutant exposure were natural logarithm transformed before input into the models. The two-pollutant models were conducted following Formula D2.

Formula D1

$$aMT6s_{ij} \sim \beta_0 + \beta_1 POL_{ij} + \beta_2 RH_{ij} + \beta_3 Temp_{ij} + \beta_4 Smoking_i + \beta_5 RI_{ij} + \beta_6 Sex_i + \beta_7 Age_i + \beta_8 BMI_i + W_i + P_i + \epsilon$$

Formula D2

$$aMT6s_{ij} \sim \beta_0 + \beta_1 POL_{ij} + \beta_2 RH_{ij} + \beta_3 Temp_{ij} + \beta_4 Smoking_i + \beta_5 RI_{ij} + \beta_6 Sex_i + \beta_7 Age_i + \beta_8 Second_POL_{ij} + \beta_9 BMI_i + W_i + P_i + \epsilon$$

D1.2 The associations of aMT6s with inflammatory cytokines

The association between aMT6s and inflammatory cytokines were assessed using LMER models following *Formula D3*.

Formula D3

$$C_{yto_{ij}} \sim \beta_0 + \beta_1 aMT6s_{ij} + \beta_2 RH_{2w_{ij}} + \beta_3 Temp_{2w_{ij}} + \beta_4 Smoking_i + \beta_5 RI_{ij} + \beta_6 Sex_i + \beta_7 Age_i + \beta_8 Batch_{ij} + \beta_9 BMI_i + W_i + P_i + \epsilon$$

D1.3 Mediation analysis

A mediation analysis was conducted to test whether aMT6s mediate the relationships between 2-week O₃ exposure and inflammatory cytokines. LMER models were used to develop mediator model and outcome model to conduct this analysis. In the mediator model (*Formula D4*), aMT6s concentration was the dependent variable. 2-week O₃ exposure was the independent variable. In the outcome model (*Formula D5*), the concentration of inflammatory cytokines was the dependent variable, and 2-week O₃ exposure concentration was dependent variable. The mediation analysis used quasi-Bayesian approximation with simulation of 5,000 times.

Formula D4

$$aMT6s_{ij} \sim \beta_0 + \beta_1 Ozone_{2w_{ij}} + \beta_2 RH_{2w_{ij}} + \beta_3 Temp_{2w_{ij}} + \beta_4 Smoking_i + \beta_5 RI_{ij} + \beta_6 Sex_i + \beta_7 Age_i + \beta_8 BMI_i + W_i + P_i + \epsilon$$

Formula D5

$$C_{yto_{ij}} \sim \beta_0 + \beta_1 Ozone_{2w_{ij}} + \beta_2 aMT6s_{ij} + \beta_3 RH_{2w_{ij}} + \beta_4 Temp_{2w_{ij}} + \beta_5 Smoking_i + \beta_6 RI_{ij} + \beta_7 Sex_i + \beta_8 Age_i + \beta_9 Batch_{ij} + \beta_{10} BMI_i + W_i + P_i + \epsilon$$

Codebook

i: the participant id number ($i= 1, 2, \dots, 53$)

j: the sample number ($j= 1,2,3$)

Cyto_{ij}: the natural logarithm-transformed concentration of inflammatory cytokines (pg/mL)

aMT6s_{ij}: the natural logarithm-transformed concentration of urinary aMT6s (ng/mL)

Pol_{ij}: the average 12-hour, 24-hour, 1-week, and 2-week personal air pollutant exposure (PM_{2.5}, O₃, NO₂, SO₂)

Second_Pol_{ij}: the co-pollutant exposure (PM_{2.5}, O₃, NO₂, or SO₂)

Ozone_2w_{ij}: the 2-week personal ozone exposure (ppb)

RH_{ij}: the average 12-hour, 24-hour, 1-week, and 2-week ambient relative humidity prior to sample collection (%).

Temp_{ij}: the average 12-hour, 24-hour, 1-week, and 2-week outdoor temperature prior to sample collection (unit: °C)

RH_2w_{ij}: the average 2-week ambient relative humidity prior to sample collection (%).

Temp_2w_{ij}: the average 2-week outdoor temperature prior to sample collection (unit: °C)

Smoking_{ij}: the current smoking status of subject (0=non-current smoker, 1=current smoker)

RI_{ij}: the respiratory infection status (0=non-respiratory infection, 1=respiratory infection)

Sex_{ij}: the sex of subject (0=male, 1=female)

Age_{ij}: the age of subject

BMI_{ij}: the body mass index of subject (Kg/m²)

W_{ij}: the day of week for biospecimen collection

Batch_{ij}: the batch factor for cytokine measurement (1=batch 1, 2= batch 2, 3=batch 3)

P_i: the individual-specific random intercept

ϵ : residual

D2. Model results

Table D1. The model results of Figure 14.

Biomarker		O ₃				PM _{2.5}			
		Effect size	CI (lower)	CI (upper)	P value	Effect size	CI (lower)	CI (upper)	P value
aMT6s	12h	-0.2	-14.5	16.5	0.98	8.0	-8.3	27.1	0.35
aMT6s	24h	-3.5	-17.9	13.4	0.66	2.6	-11.6	19.1	0.73
aMT6s	1w	-16.8	-38.7	13.1	0.24	17.2	-3.6	42.4	0.11
aMT6s	2w	-26.2	-43.9	-2.8	0.03	20.1	1.7	41.8	0.03
		NO ₂				SO ₂			
aMT6s	12h	-0.6	-11.2	11.3	0.91	11.7	-3.6	29.6	0.14
aMT6s	24h	2.3	-6.9	12.4	0.63	10.8	-5.8	30.5	0.21
aMT6s	1w	19.2	-2.6	45.7	0.09	22.7	-1.2	52.5	0.06
aMT6s	2w	14.1	-2.9	34.0	0.11	59.4	14.8	121.4	0.01

Table D2. The model results of Figure 15.

aMT6s				
Biomarker	Effect size	CI (lower)	CI (upper)	P value
IL-1 β	-17.8	-31.4	-1.6	0.03
IL-2	-10.6	-23.4	4.4	0.16
IL-6	-8.3	-23.4	9.8	0.34
IL-8	-21.6	-35.2	-5.2	0.01
IL-17A	-18.8	-32.0	-3.0	0.02
IFN- γ	-27.2	-42.0	-8.7	0.01
TNF- α	-17.6	-28.2	-5.6	0.01
IL-4	-15.5	-50.2	43.5	0.53
IL-10	0.7	-28.3	41.4	0.97

D3. Sensitivity analyses

Sensitivity analyses were conducted for the associations shown in the main text in dataset removing either measurements from current smokers or measurements from participants who reported respiratory infection 1-week prior to the biospecimen collection following *Formula D1, D2, D3, D4, D5*.

D3.1 Excluding measurements for which participants reported respiratory infection (Excluding 13 measurements from 11 subjects)

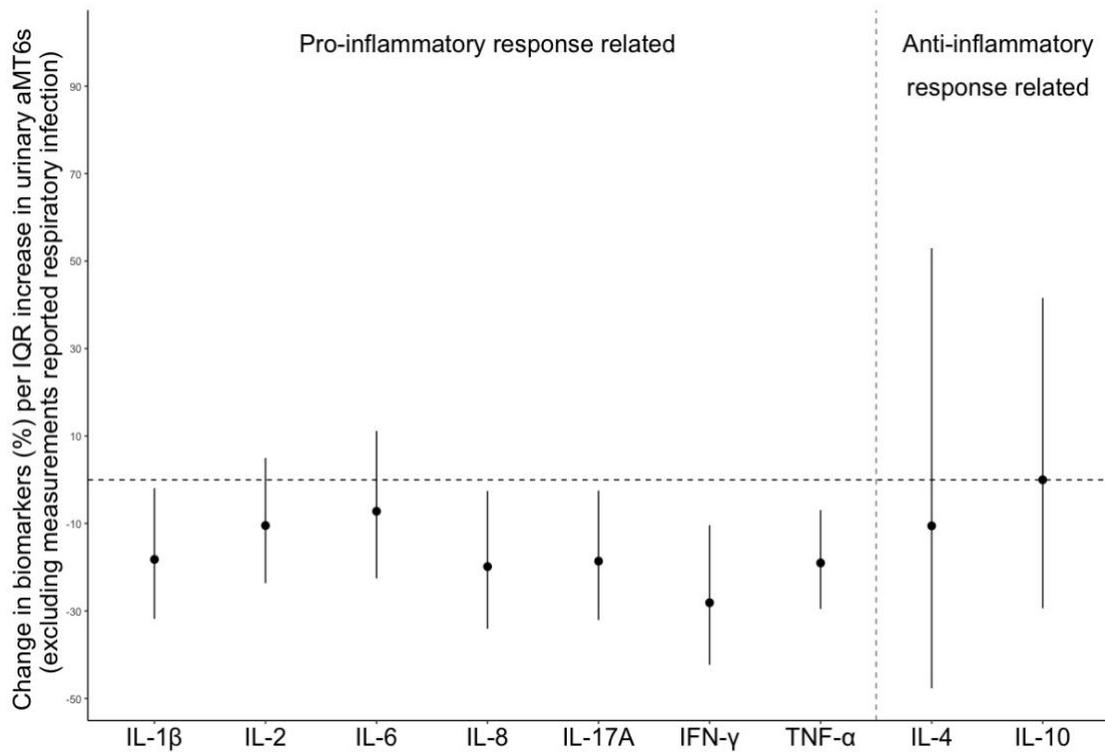


Figure D1. Estimated means and 95% confident intervals for change in inflammatory cytokines (%) per IQR increase in aMT6s (Excluding measurements for which participants reported respiratory infection).

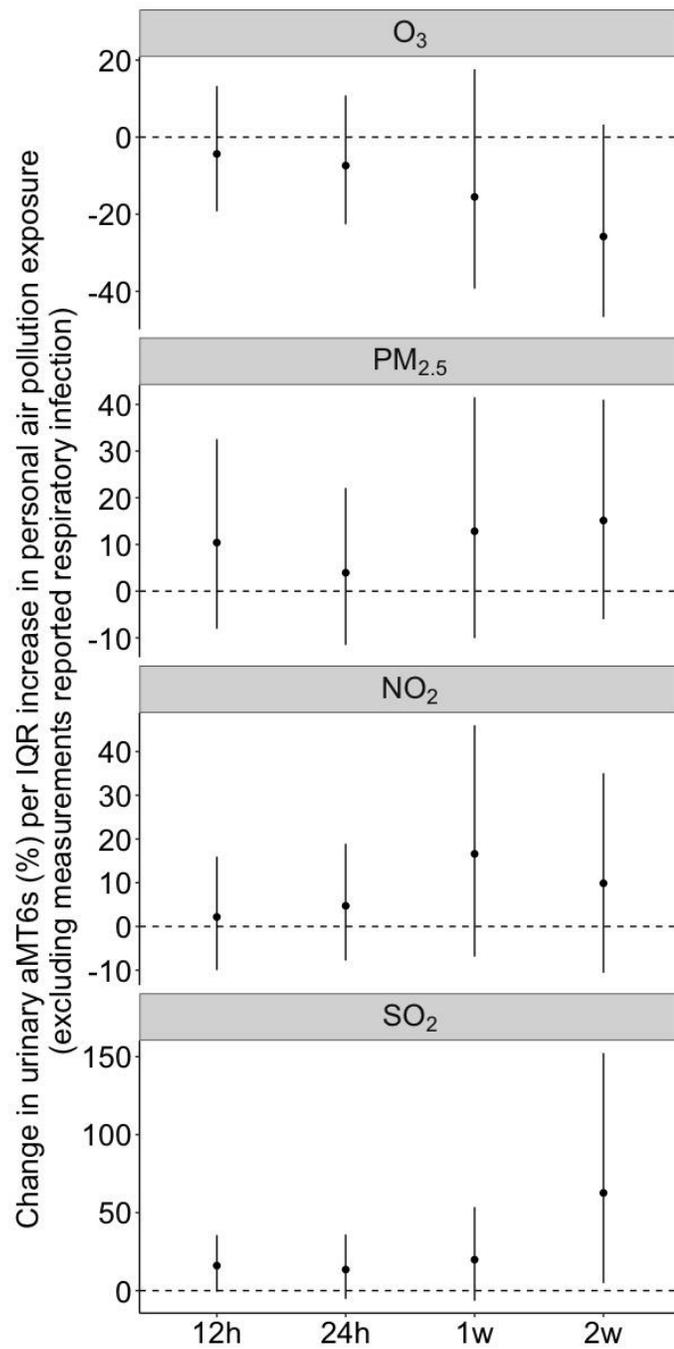


Figure D2. Estimated means and 95% confident intervals for change in urinary aMT6s (%) per IQR increase in 12-hour (12h), 24-hour (24h), 1-week (1w), and 2-week (2w) pollutant exposure (Excluding measurements for which participants reported respiratory infection).

Table D3. The proportion of the total effects of 2-week O₃ exposure on inflammatory cytokine mediated by aMT6s (Excluding measurements for which participants reported respiratory infection).

	Proportion (%)	95% CI (%)	P-value
IL-1 β	15.4	(0.3 – 76.2)	0.04
IL-8	6.2	(-4.1 – 31.1)	0.19
IL-17A	7.6	(-1.8 – 25.3)	0.08
IFN- γ	8.3	(0.5 – 39.2)	0.03
TNF- α	19.9	(0.6 – 139.5)	0.04

D3.2 Excluding current smoker (Excluding 27 measurements from 11 subjects).

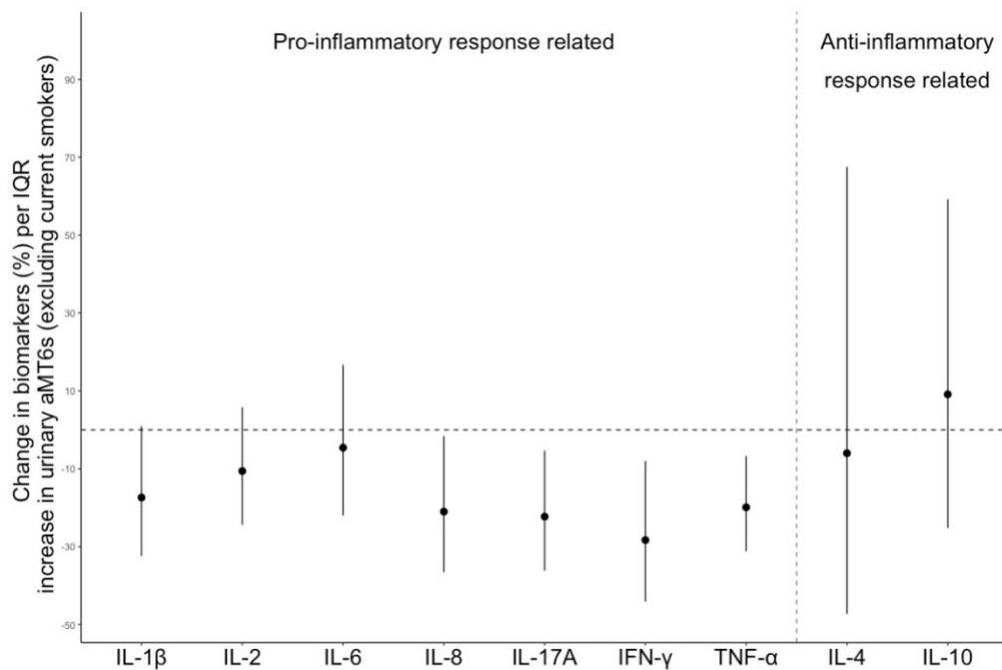


Figure D3. Estimated means and 95% confident intervals for change in inflammatory cytokines (%) per IQR increase in aMT6s (Excluding current smoker).

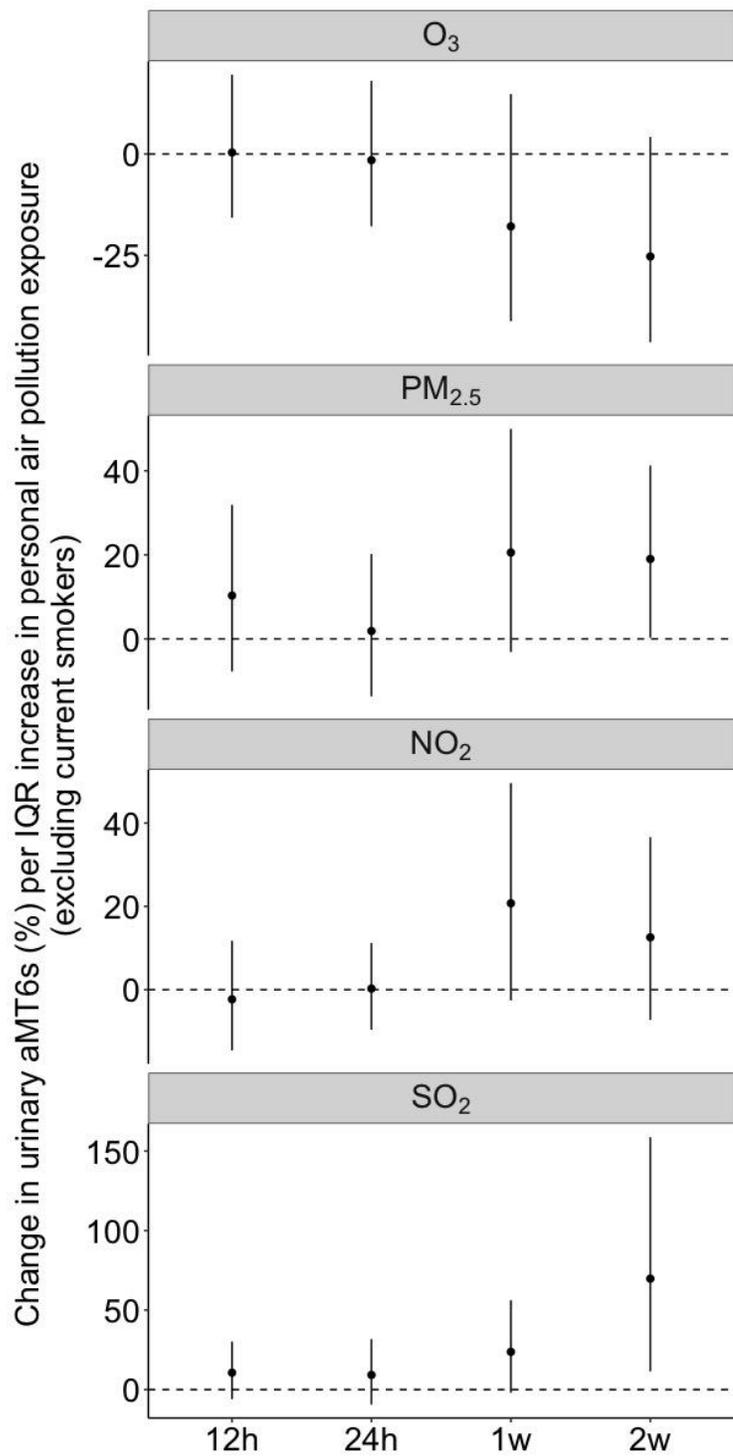


Figure D4. Estimated means and 95% confident intervals for change in urinary aMT6s (%) per IQR increase in 12-hour (12h), 24-hour (24h), 1-week (1w), and 2-week (2w) pollutant exposure (Excluding current smoker).

Table D4. The proportion of the total effects of 2-week O₃ exposure on inflammatory cytokine mediated by aMT6s (Excluding current smoker).

	Proportion (%)	95% CI (%)	P-value
IL-1 β	13.9	(-3.3 – 95.2)	0.09
IL-8	7.9	(-3.6 – 37.6)	0.18
IL-17A	13.4	(-1.9 – 57.7)	0.08
IFN- γ	11.6	(0.1 – 45.4)	0.04
TNF- α	26.1	(-137.7 – 251.8)	0.14

D3.3 Co-pollutant models

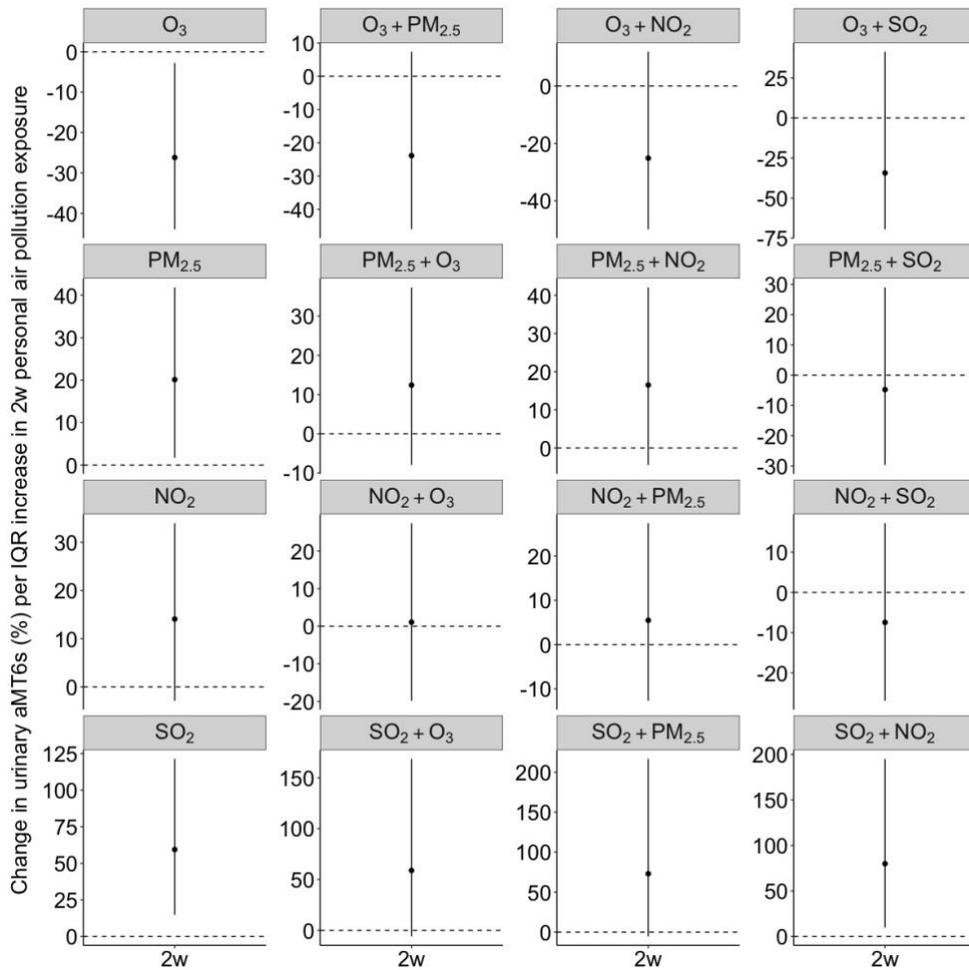


Figure D5. Estimated means and 95% confident intervals for change in urinary aMT6s (%) per IQR increase in 2-week (2w) pollutant exposure (Co-pollutant models).

D3.4 Investigate the prolonged effects of temperature

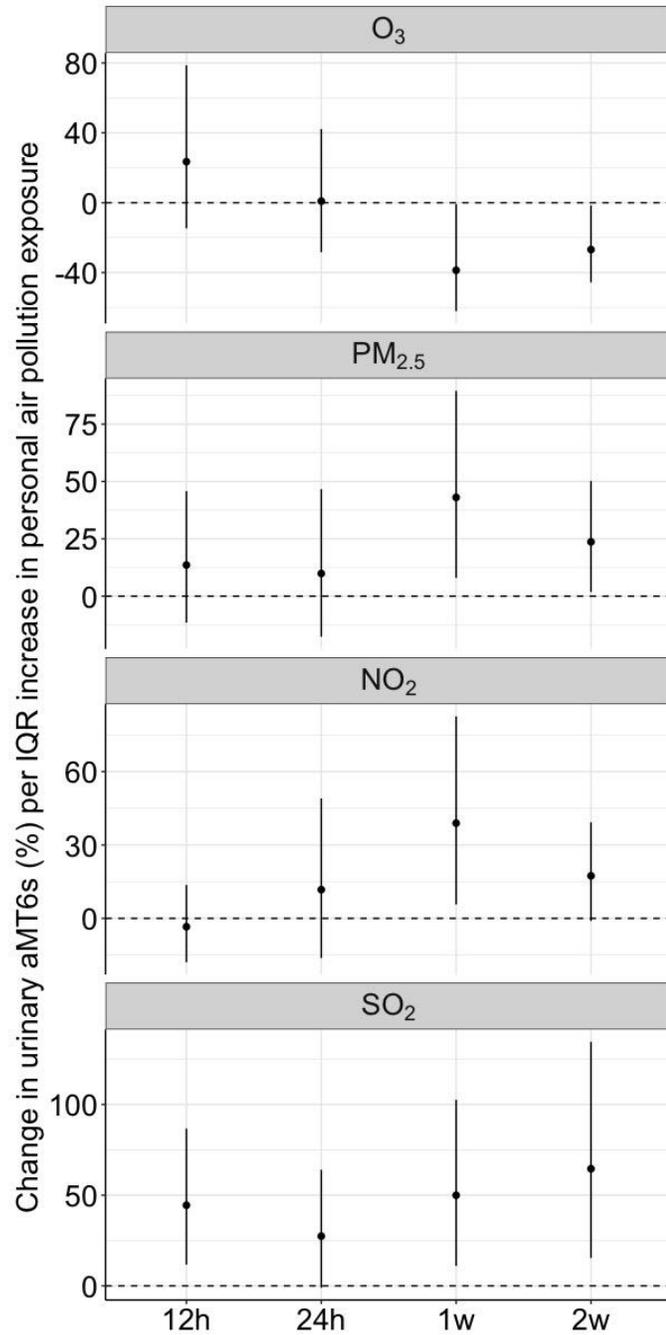


Figure D6. Estimated means and 95% confident intervals for change in urinary aMT6s (%) per IQR increase in 12-hour (12h), 24-hour (24h), 1-week (1w), and 2-week (2w) pollutant exposure (Controlling for all temperature averaged over 12h, 24h, 1w, and 2w in models).

Appendix E

Appendix E contains the supporting information for Chapter 6, published online from He, L.; Li, Z.; Teng, Y.; Cui, X.; Barkjohn, K. K.; Norris, C.; Fang, L.; Lin, L.; Wang, Q.; Zhou, X.; Hong, J.; Li, F.; Zhang, Y.; Schauer, J. J.; Black, M.; Bergin, M.; Zhang, J., Associations of personal exposure to air pollutants with airway mechanics in children with asthma. *Environment International*. 2020, 138 (Publisher: Elsevier).

E1. Personal air pollutant exposure assessment

E1.1 Air pollutant measurement

PM_{2.5} and O₃ were simultaneously measured both in children's bedrooms and outside a window of their homes during the study period. These pollutants were continuously measured by an integrated sensor box equipped with Plantower PMS3003 sensor for PM_{2.5} and Alphasense sensor (OX-A4) for O₃. The hourly averages of pollutant concentrations were generated by the sensors. These sensors, validated in Beijing, Shanghai, and other cities previously,^{194, 199-201} were field calibrated in Shanghai before the start of the study and at the end of the study to account for any drift in sensor function over the duration of the study. Ambient hourly average of PM_{2.5}, O₃, temperature, and relative humidity during the study period were measured at the nearest government monitoring station (Qingpu Environmental Monitoring Station, Shanghai) and shown in Table E1.

Missing air pollutant data measured by the governmental monitoring sites were extrapolated using the average of the concentrations measured by sensors located directly outside the homes at the same time. Missing air pollutant data measured by the sensors located directly outside the homes were extrapolated using the concentration measured by the nearest home's outdoor sensor at the same time. Missing air pollutant data measured by the sensors located in the bedroom were extrapolated using the concentration measured by the sensor directly outside the home, multiplied by the appropriate I/O ratio shown in Table E2. During the filtration period, we calculated the home specific I/O ratio for three-filter filtration and coarse-filter filtration period, respectively, based on the existing measurements from indoor and outdoor sensors.

In this study, the ambient air pollutant concentrations were calculated by averaging the pollutant concentrations measured by all the outdoor sensors and the governmental monitoring station. Air pollutant concentrations in other indoor environments, in car/taxi, in the subway, and at other outdoor locations, were calculated by multiplying the ambient air pollutant concentrations by the appropriate I/O ratio shown in Table E2. Pollutant concentrations in the bedroom and other rooms in the home were assumed to be similar, and the air pollutant concentrations measured in the bedroom were used for both of these environments.

Table E1. Ambient pollutant level, temperature, and relative humidity.

	Value
PM _{2.5} , mean ± SD [range], µg/m ³	56.8 ± 28.9 [3.0-185.0]
O ₃ (Maximum daily 8-hour average), mean± SD [range], ppb	54.7 ± 17.9 [12.6-93.4]
Temperature, mean± SD [range], °C	11.3 ± 4.3 [2.3-24.0]
Relative humidity, mean± SD [range], %	73.7 ± 18.6 [20.7-99.3]

Table E2. I/O ratio for each microenvironment.

	I/O Ratio	
	PM _{2.5}	O ₃
Bedroom (window closed, during the filtration period)	Mean Home specific I/O ratio ± SD (0.44 ± 0.60)	Mean Home specific I/O ratio ± SD (0.35 ± 0.42)
Bedroom (window closed, during the no filtration period)	0.8	0.35
Bedroom (window opened)	1	0.85
Other indoor environment outside home (window closed)	0.8	0.35
Other indoor environment outside home (window opened)	1	0.85
Car/Taxi	0.8	0.4
Subway	0.8	0.35
Walk/ Bike/outdoor exercise	1	1

Table E3. Time activity in different microenvironments.

	Value
Bedroom, hour/day	12.2 ± 2.5
Other room in home, hour/day	3.6 ± 2.5
Car/Taxi, hour/day	0.1 ± 0.3
Subway, hour/day	0.002 ± 0.008
Other indoor environment outside home (e.g., classroom, cafeteria, and other family members' and friends' homes), hour/day	8.1 ± 2.1
Outdoor, hour/day	2.3 ± 1.4

E1.2 Time activity pattern

During each clinical visit, participants were asked to record their time-activity patterns for the two weeks preceding the visit on a time activity questionnaire. On the time activity questionnaire, participants would indicate their location during each hour by selecting among the bedroom, other rooms in home, a classroom, a car/taxi, in the subway, other indoor microenvironments, or outdoors (walking, biking, or doing exercise outdoors). The descriptive statistics of time activity data in different microenvironments were shown in Table E3. If the subjects were indoors, they were asked to indicate whether the window of the room they were in was open.

E1.3 Personal air pollutant exposure calculation

The personal air pollutant exposure at each hour was calculated by multiplying the location factor (0=not in the location, 1=in the location) with air pollutant level at each of corresponding microenvironment during the corresponding time period and summing them up (*Formula E1*). Based on the hourly exposure, we could calculate the 2-week average air pollutant exposure and 24-hour average air pollutant exposure zero to six days prior to each of the clinical visits.

Formula E1

$$EXP_{ijt} = \sum_{k=1}^6 C_{ijtk} * L_{ijtk}$$

Code Book

EXP_{ijt} : the hourly personal air pollutant exposure for study participant i at hour t at visit j .

k : the microenvironments (1: bedroom, 2: other room in home, 3: car, 4: subway, 5: other indoor environment outside home, 6: outdoor)

i : the participant id number ($i=1, 2, \dots, 43$)

j : the visit ($j= 1, 2, 3, 4$)

t : the hour before health indicators measurement up to 2 weeks ($t= 1, 2, \dots, 336$)

C_{ijt} : the air pollutant concentration of microenvironment k where this study participant i at hour t at visit j

L_{ijt} : the location factor for the microenvironment k where this study participant i at hour t at visit j (0: not in the microenvironment or 1: in the microenvironment)

E2. Statistical analysis

E2.1 Health outcomes in relation to filtration status

We used linear mixed-effects regression (LMER) models to examine the relationship between filtration status and health outcomes and to investigate the effects of changes in pollutant exposure caused by the operation of air purifiers following *Formula E2*. We used *lme4* and *lmeTest* packages of the R software (version 3.6.1) to conduct the analysis. In these models, a health outcome was the dependent variable, filtration status (no filtration versus coarse+HEPA+activated carbon filters versus coarse filters) was the independent variable; the fixed-effects covariates included changes in 2-week average personal PM_{2.5} and O₃ exposure, 2-week average ambient temperature and relative humidity, sex, age, baseline eosinophil count, upper respiratory tract infection status, opioid cough suppressant usage, dust mite allergy status, sleep duration, asthma exacerbation status, inhaled corticosteroids usage (whether or not used inhaled corticosteroids during the two weeks prior to each of the clinical visits), and travel status (whether or not traveled during the two weeks prior to each of the clinical visits). We controlled for subject ID and the day of week for clinical visit as random intercepts. From the model output, we calculated percent change (and 95% confidence interval) in the outcome following the use of the three filters together or the use of only the coarse filter in reference to following the absence of any filters.

Formula E2

$$Y_{ij} \sim \beta_0 + \beta_1 Fil_{ij} + \beta_2 PM2.5_{2w_{ij}} + \beta_3 O3_{2w_{ij}} + \beta_4 RH_{2w_{ij}} + \beta_5 Temp_{2w_{ij}} + \beta_6 Sex_i + \beta_7 Age_i + \beta_8 EOS_i \\ + \beta_9 Fever_{ij} + \beta_{10} Opioid_{ij} + \beta_{11} dustmite_i + \beta_{12} Sleep_{ij} + \beta_{13} ICS_{ij} + \beta_{14} Travel_{ij} + \beta_{15} E_{ij} \\ + P_i + Wday_{ij} + \varepsilon$$

E2.2 Exposure-response relationships

LMER models were constructed to determine the exposure-response association between air pollutant exposure and health indicators following *Formula E3*. The co-pollutant models were shown as described in *Formula E4*. The dependent variable in the model was each of the pulmonary health indicators, and the independent variable was each of the personal air pollutant exposures measured zero to six days prior to the clinical visits. Some biomarkers and air pollutant exposure were natural logarithm-transformed due to skewed distributions. The models were adjusted for fixed-effect covariates including ambient temperature and relative humidity averaged over the same time with pollution exposure, sex, age, baseline eosinophil count, upper respiratory tract infection status, opioid cough suppressant usage, dust mite allergy status, sleep duration, asthma exacerbation status, inhaled corticosteroids usage, and travel status. We controlled for subject ID and the day of the week for clinical visit as random intercepts. From the model output, we calculated percent change (and 95% confidence interval) of the outcome associated with an IQR increase in personal pollutant exposure. Based on the results from the first set of the models (Section E2.1), we deemed it not

appropriate to include the data measured following the use of only the coarse filter.

Hence, the data from three visits per person were used in the analysis of exposure-response relationships.

Formula E3

$$Y_{ij} \sim \beta_0 + \beta_1 Pol_{ij} + \beta_2 RH_{ij} + \beta_3 Temp_{ij} + \beta_4 Sex_i + \beta_5 Age_i + \beta_6 EOS_i + \beta_7 Fever_{ij} + \beta_8 Opioid_{ij} + \beta_9 dustmite_i \\ + \beta_{10} Sleep_{ij} + \beta_{11} ICS_{ij} + \beta_{12} Travel_{ij} + \beta_{13} E_{ij} + P_i + Wday_{ij} + \varepsilon$$

Formula E4

$$Y_{ij} \sim \beta_0 + \beta_1 Pol_{ij} + \beta_2 RH_{ij} + \beta_3 Temp_{ij} + \beta_4 Sex_i + \beta_5 Age_i + \beta_6 EOS_i + \beta_7 Fever_{ij} + \beta_8 Opioid_{ij} + \beta_9 dustmite_i \\ + \beta_{10} Sleep_{ij} + \beta_{11} ICS_{ij} + \beta_{12} Travel_{ij} + \beta_{13} Second_Pol_{ij} + \beta_{14} E_{ij} + P_i + Wday_{ij} + \varepsilon$$

Codebook

i: the participant id number (*i*= 1, 2, ..., 43)

j: the sample number for Formula E2 (*j*= 1, 2, 3, 4); the sample number for Formula E3 and 4 (*j*= 1, 2, 3)

Fil_{*ij*}: the filtration status (no filtration vs HEPA + activated carbon + coarse filter vs coarse filter)

Y_{*ij*}: the concentration of pulmonary health indicators, including Z₅, R₅, R₂₀, R_{5-R20}, X₅, Fres, FEV₁, FVC, PEF, FEF₂₅₋₇₅, FEV₁/FVC, and FeNO. Among them, FEV₁, FVC, FEF₂₅₋₇₅, FEV₁/FVC, and FeNO were nature logarithm transformed (unit: Z₅ (cm H₂O/L/s), R₅ (cm H₂O/L/s), R₂₀ (cm H₂O/L/s), R_{5-R20} (cm H₂O/L/s), X₅ (cm H₂O/L/s), Fres (Hz), FEV₁ (L), FVC (L), PEF (L/s), FEF₂₅₋₇₅ (L/s), FEV₁/FVC (%), and FeNO (ppb))

Pol_{*ij*}: the 24-hour average personal PM_{2.5} exposure and the maximum 8-hour average personal O₃ exposure measured zero to six days prior to the clinical visit (nature logarithm transformed) (unit: PM_{2.5}: µg/m³ and O₃: ppb)

PM2.5_2w_{*ij*}: the 2-week average personal PM_{2.5} exposure (unit: µg/m³)

O3_2w_{*ij*}: the 2-week average personal O₃ exposure (unit: ppb)

Second_Pol_{*ij*}: the personal O₃ exposure controlled as the second pollutant averaged with the same time frame as the Pol_{*ij*} (unit: ppb)

RH_{*ij*}: the 24-hour average ambient relative humidity measured zero to six days prior to health indicators measurement (unit: %)

RH_2w_{ij}: the ambient relative humidity averaged over 2-week prior to health indicators measurement (unit: %)

Temp_{ij}: the 24-hour average ambient temperature measured zero to six days prior to health indicators measurement, a natural spline with degrees of freedom equals 3 was applied (unit: °C)

Temp_2w_{ij}: the ambient temperature averaged over 2-week prior to health indicators measurement (unit: °C)

Age_i: the subjects' age (unit: year)

Sex_i: the subjects' sex (0=male, 1=female)

EOS_i: the baseline eosinophil number (unit: / μ L)

Dustmite_i: the baseline allergic status to dust mite (0=non-allergic, 1=allergic)

ever_{ij}: the upper respiratory tract infection status during 2 weeks prior to the clinical visit (0=non-respiratory infection, 1=respiratory infection)

Opioid_{ij}: the opioid cough suppressant usage status during 2 weeks prior to the clinical visit (0=non used, 1= used)

Sleep_{ij}: the sleeping duration for the night prior to the clinical visit (unit: hour)

ICS_{ij}: the status of using inhaled corticosteroids during 2-weeks prior to each of the clinical visit (0=not-used, 1=used)

Travel_{ij}: the status of traveling during 2-weeks prior to each of the clinical visit (0=non-travel, 1=travel)

Wday_{ij}: the random intercept for the day of week for clinical visit.

P_i: the individual-specific random intercept.

E_{ij}: the asthma exacerbation status (0=non-exacerbation, 1=exacerbation)

ε : residual.

E3. Spearman correlations among air pollutant exposures, relative humidity, and temperature.

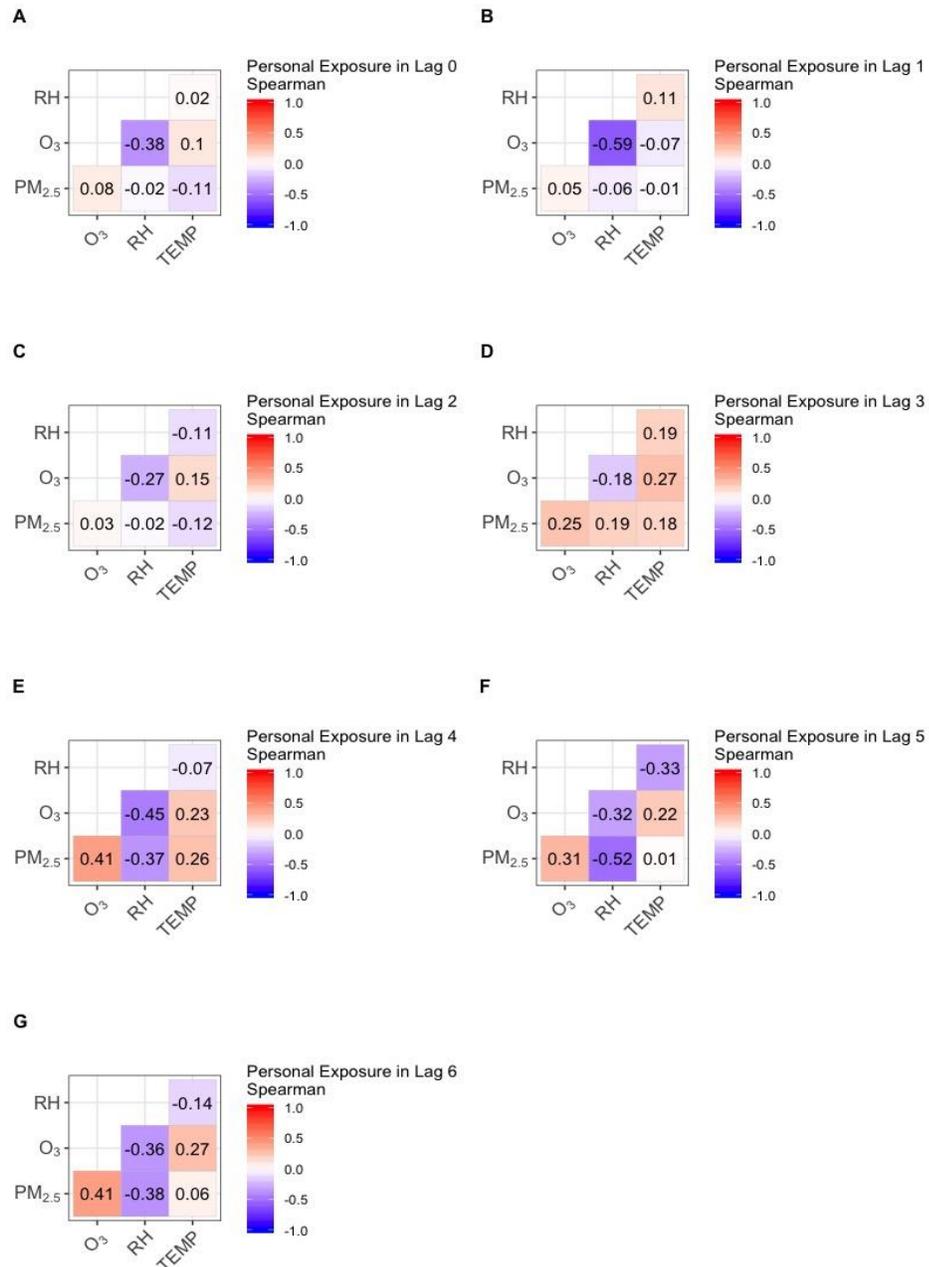


Figure E1. The Spearman correlations among air pollutant exposures, relative humidity, and temperature. RH: ambient relative humidity, TEMP: ambient temperature

E4. Sensitivity analyses

E4.1 Exposure-response relationship (Only no-filtration visits)

If concentration of volatile organic compounds (VOCs) were largely different between three-filter filtration and no-filtration period, this could have confounded the exposure-response relationship. Therefore, we tested the relationships between PM_{2.5} exposure and indicators of pulmonary physiology only in the two no-filtration visits following *Formula E3*, to test the robustness of findings in the main analysis. As shown in Figure E2, we found that the increase in 24-hour average PM_{2.5} exposure measured one day prior to the clinical visit was associated with significantly decreases in FEV₁ and FVC. This analysis, conducted to reduce the effects of potential confounding of co-pollutant exposures, could support the robustness of findings in the main analysis. However, since the small day-to-day changes (without filtration manipulation) in PM_{2.5} limited the statistical powers, we found associations of increased airway impedance (Z₅) and inflammation (FeNO) with increasing PM_{2.5} exposures measured one-day prior, although these associations were not statistically significant.

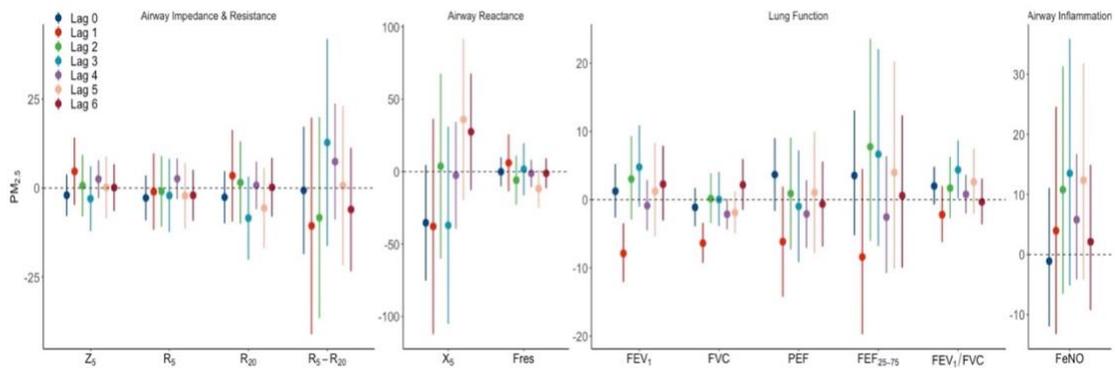


Figure E2. Estimated means and 95% confident intervals for change in biomarkers (%) with one IQR increase in 24-hour average $PM_{2.5}$ personal exposure in zero to six days prior to health outcome measurement (lag 0-6) only in the two no-filtration visits. Z_5 =airway impedance measured at 5Hz; R_5 =airway resistance measured at 5Hz; R_{20} =airway resistance measured at 20Hz; R_5-R_{20} =difference between airway resistance measured at 5Hz and 20Hz; X_5 =airway reactance measured at 5Hz; F_{res} =resonant frequency; FEV_1 =forced expiratory volume in first second; FVC =forced vital capacity; PEF : peak expiratory flow; FEF_{25-75} =the average forced expiratory flow during 25% to 75% of FVC; FEV_1/FVC = the ratio of FEV_1 and FVC ; $FeNO$ =fractional exhaled nitric oxide.

E4.2 Co-pollutant models

Co-pollutant models, following *Formula E4*, were developed to examine whether the PM_{2.5} exposure-response relationship obtained in the single-pollutant models can be retained after controlling for O₃ as the co-pollutant. The exposure-response relationship between personal PM_{2.5} exposure and pulmonary health indicators were not remarkably different with respect to the significance or effect size after controlling for O₃ (Figure E3). The relationships of Z₅ with personal PM_{2.5} exposure measured in the three days and four days prior changed from non-significant to significant. Similar changes were also found in the relationship between R₅ and personal PM_{2.5} exposure measured in the three days prior and the relationship between FEF₂₅₋₇₅ and PM_{2.5} exposure measured six days prior. Similarly, controlling for O₃ as the co-pollutant in the models does not remarkably change the associations between the health outcomes and PM_{2.5} exposure in the group with high or low blood eosinophil counts in terms of both statistical significance and effect sizes (Figure E4).

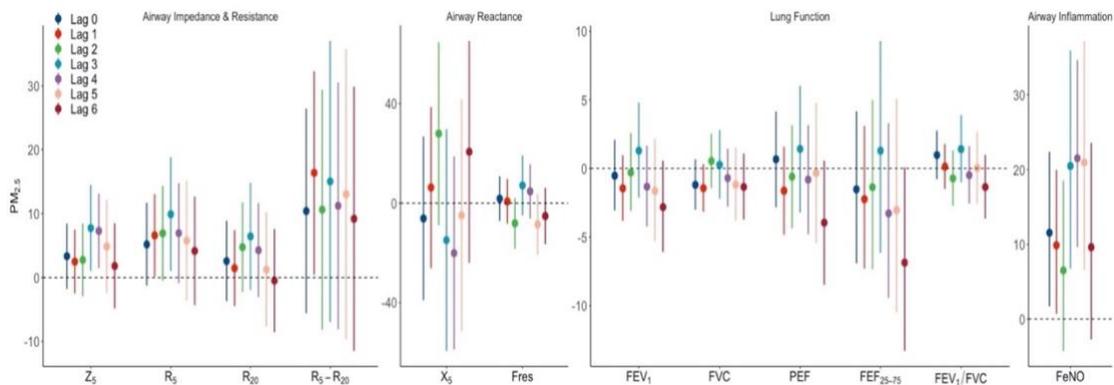


Figure E3. Estimated means and 95% confident intervals for change in biomarkers (%) with one IQR increase in 24-hour average PM_{2.5} personal exposure in zero to six days prior to health outcome measurement (lag 0-6) with O₃ controlled as a co-pollutant in the models. Z₅=airway impedance measured at 5Hz; R₅=airway resistance measured at 5Hz; R₂₀=airway resistance measured at 20Hz; R₅-R₂₀=difference between airway resistance measured at 5Hz and 20Hz; X₅=airway reactance measured at 5Hz; Fres=resonant frequency; FEV₁=forced expiratory volume in first second; FVC=forced vital capacity; PEF: peak expiratory flow; FEF₂₅₋₇₅=the average forced expiratory flow during 25% to 75% of FVC; FEV₁/FVC= the ratio of FEV₁ and FVC; FeNO=fractional exhaled nitric oxide.

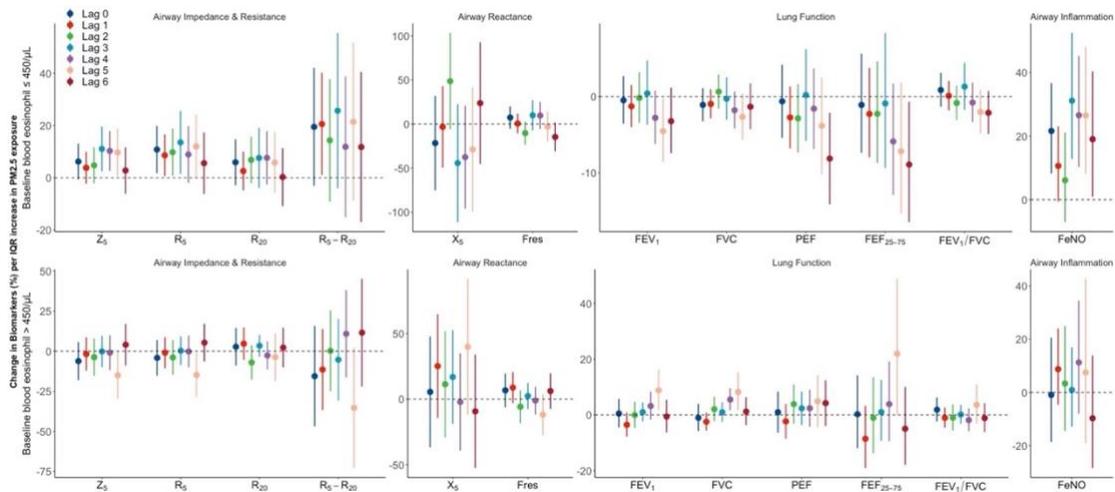


Figure E4. Estimated means and 95% confident intervals for change in biomarkers (%) with one IQR increase in 24-hour average PM_{2.5} and O₃ personal exposure in zero to six days prior to health outcome measurement (lag 0-6). Stratified by blood eosinophil number (cutoff point 450/ μ L) Z₅=airway impedance measured at 5Hz; R₅=airway resistance measured at 5Hz; R₂₀=airway resistance measured at 20Hz; R₅-R₂₀=difference between airway resistance measured at 5Hz and 20Hz; X₅=airway reactance measured at 5Hz; Fres=resonant frequency; FEV₁=forced expiratory volume in first second; FVC=forced vital capacity; PEF: peak expiratory flow; FEF₂₅₋₇₅=the average forced expiratory flow during 25% to 75% of FVC; FEV₁/FVC= the ratio of FEV₁ and FVC; FeNO=fractional exhaled nitric oxide.

E4.3 Excluding measurements of participants who traveled or used inhaled corticosteroids during the 2-weeks prior to the clinical visits.

Sensitivity analyses were conducted for the associations shown in the main content in a dataset excluding measurements of participants who traveled or used inhaled corticosteroids during the two-weeks prior to each of the clinical visits following Formula E3. As shown in Figure E5-E6, no noticeable changes were found for these associations in the new dataset in terms of statistical significance or effect sizes, compared to the results in the main text.

E4.3.1 Excluding participants traveled: removed 15 measurements from 14 participants.

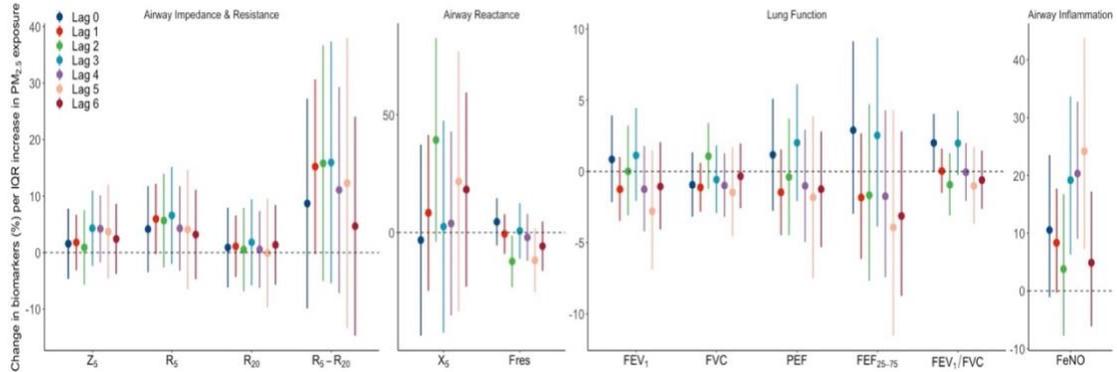


Figure E5. Estimated means and 95% confident intervals for change in biomarkers (%) with one IQR increase in 24-hour average PM_{2.5} personal exposure in zero to six days prior to health outcome measurement (lag 0-6) by excluding participants traveled during the 2-week prior to the clinical visit. Z_5 =airway impedance measured at 5Hz; R_5 =airway resistance measured at 5Hz; R_{20} =airway resistance measured at 20Hz; $R_5 - R_{20}$ =difference between airway resistance measured at 5Hz and 20Hz; X_5 =airway reactance measured at 5Hz; F_{res} =resonant frequency; FEV_1 =forced expiratory volume in first second; FVC =forced vital capacity; PEF : peak expiratory flow; FEF_{25-75} =the average forced expiratory flow during 25% to 75% of FVC; FEV_1/FVC = the ratio of FEV_1 and FVC ; $FeNO$ =fractional exhaled nitric oxide.

E4.3.2 Excluding participants used inhaled corticosteroids: removed 22 measurements from 12 participants.

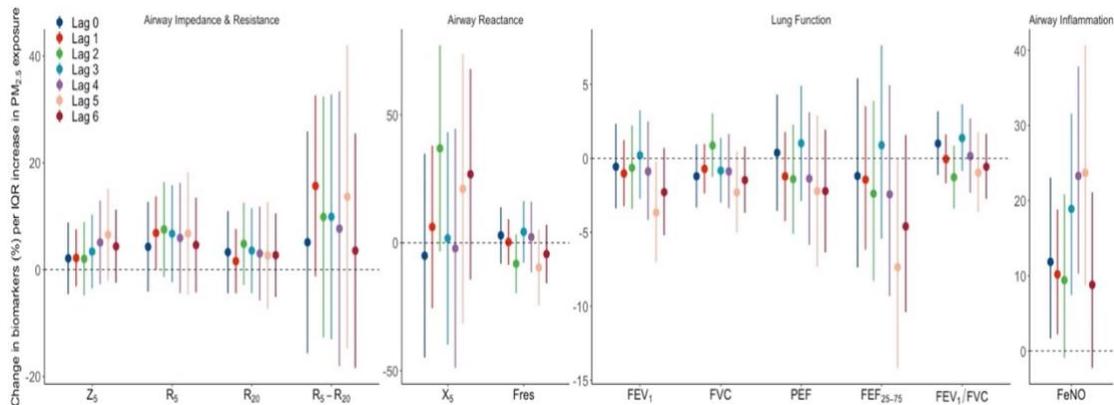


Figure E6. Estimated means and 95% confident intervals for change in biomarkers (%) with one IQR increase in 24-hour average PM_{2.5} personal exposure in zero to six days prior to health outcome measurement (lag 0-6) by excluding participants used inhaled corticosteroids during the 2-week prior to the clinical visit. Z₅=airway impedance measured at 5Hz; R₅=airway resistance measured at 5Hz; R₂₀=airway resistance measured at 20Hz; R₅-R₂₀=difference between airway resistance measured at 5Hz and 20Hz; X₅=airway reactance measured at 5Hz; F_{res}=resonant frequency; FEV₁=forced expiratory volume in first second; FVC=forced vital capacity; PEF: peak expiratory flow; FEF₂₅₋₇₅=the average forced expiratory flow during 25% to 75% of FVC; FEV₁/FVC= the ratio of FEV₁ and FVC; FeNO=fractional exhaled nitric oxide.

E4.4 Exposure-response relationship (Including all 4 visits)

The PM_{2.5} exposure-response relationships including all the clinical visits are shown in Figure E7. We found that day-to-day changes in personal PM_{2.5} exposure was associated with decreased lung function and increased airway resistance and airway inflammation, supporting the findings of the main analyses.

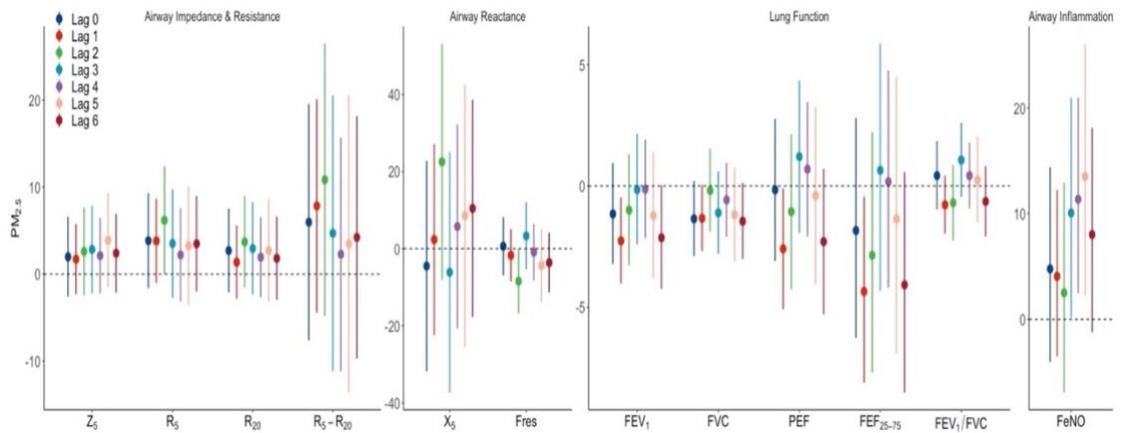


Figure E7. Estimated means and 95% confident intervals for change in biomarkers (%) with one IQR increase in 24-hour average $PM_{2.5}$ personal exposure in zero to six days prior to health outcome measurement (lag 0-6) including all visits. Z_5 =airway impedance measured at 5Hz; R_5 =airway resistance measured at 5Hz; R_{20} =airway resistance measured at 20Hz; R_5-R_{20} =difference between airway resistance measured at 5Hz and 20Hz; X_5 =airway reactance measured at 5Hz; F_{res} =resonant frequency; FEV_1 =forced expiratory volume in first second; FVC =forced vital capacity; PEF : peak expiratory flow; FEF_{25-75} =the average forced expiratory flow during 25% to 75% of FVC ; FEV_1/FVC = the ratio of FEV_1 and FVC ; $FeNO$ =fractional exhaled nitric oxide.

E5. Detailed model results

Table E4. Estimated means and 95% confident intervals for change in biomarkers (%) measured from after using no air purifier to using either air purifier with all the three filters or with only the coarse filter (For Figure 18).

Health Outcomes	Filtration Status	Mean Changes (%)	95% CI	p-value
Z ₅ , cmH ₂ O/(L/s)	Coarse+HEPA+Activated Carbon Filters	-0.32	-13.1-12.4	0.96
	Coarse Filter	8.4	-0.2-16.9	0.055
R ₅ , cmH ₂ O/(L/s)	Coarse+HEPA+Activated Carbon Filters	-2.1	-17.9-13.7	0.80
	Coarse Filter	9.7	-0.9-20.3	0.073
R ₂₀ , cmH ₂ O/(L/s)	Coarse+HEPA+Activated Carbon Filters	-8.7	-22.4-5.0	0.21
	Coarse Filter	-0.081	-9.3-9.2	0.99
R ₅ -R ₂₀ , cmH ₂ O/(L/s)	Coarse+HEPA+Activated Carbon Filters	6.1	-34.0-46.2	0.76
	Coarse Filter	27.6	0.5-54.7	0.046
X ₅ , cmH ₂ O/(L/s)	Coarse+HEPA+Activated Carbon Filters	-1.7	-82.7-79.2	0.97
	Coarse Filter	-27.7	-82.6-27.3	0.32
Fres, Hz	Coarse+HEPA+Activated Carbon Filters	-3.4	-26.1-19.3	0.77
	Coarse Filter	7.8	-7.4-23.0	0.31
FEV ₁ , L	Coarse+HEPA+Activated Carbon Filters	-2.3	-8.2-3.9	0.45
	Coarse Filter	-0.57	-4.6-3.6	0.79
FVC, L	Coarse+HEPA+Activated Carbon Filters	-4.2	-8.4-0.27	0.065
	Coarse Filter	-2.2	-5.1-0.78	0.14
PEF, L/S	Coarse+HEPA+Activated Carbon Filters	-3.1	-11.5-5.4	0.48
	Coarse Filter	2.6	-3.1-8.3	0.36
FEF ₂₅₋₇₅ , L/s	Coarse+HEPA+Activated Carbon Filters	0.73	-12.2-15.5	0.92
	Coarse Filter	2.8	-6.3-12.7	0.56
FEV ₁ /FVC	Coarse+HEPA+Activated Carbon Filters	2.6	-1.7-7.0	0.24
	Coarse Filter	2.1	-0.73-5.0	0.14
FeNO, ppb	Coarse+HEPA+Activated Carbon Filters	-1.8	-23.7-26.4	0.89
	Coarse Filter	12.5	-4.9-33.0	0.17

Table E5. Estimated means and 95% confident intervals for change in biomarkers (%) with one IQR increase in 24-hour average PM_{2.5} in zero to six days prior to health outcome measurement (lag 0-6) (For Figure 19).

Health Outcomes	PM _{2.5} Exposures	IQR, $\mu\text{g}/\text{m}^3$	Mean Changes (%)	95% CI	p-value
Z ₅ , cmH ₂ O/(L/s)	Lag0	27.1	3.2	-2.4-8.8	0.26
	Lag1	30.3	2.4	-2.4-7.3	0.32
	Lag2	32.0	2.7	-2.9-8.2	0.38
	Lag3	22.8	4.2	-1.6-10.0	0.15
	Lag4	29.2	4.2	-0.7-9.2	0.092
	Lag5	38.9	5.4	-2.3-13.1	0.17
	Lag6	34.6	1.9	-4.1-7.9	0.53
R ₅ , cmH ₂ O/(L/s)	Lag0	27.1	5.1	-1.9-12.2	0.15
	Lag1	30.3	6.3	0.10-12.5	0.048
	Lag2	32.0	7.0	-0.3-14.2	0.060
	Lag3	22.8	6.6	-1.2-14.4	0.094
	Lag4	29.2	4.8	-1.7-11.3	0.15
	Lag5	38.9	6.4	-3.7-16.7	0.21
	Lag6	34.6	3.5	-4.2-11.1	0.37
R ₂₀ , cmH ₂ O/(L/s)	Lag0	27.1	2.6	-4.2-9.5	0.44
	Lag1	30.3	1.4	-4.4-7.1	0.64
	Lag2	32.0	4.5	-2.3-11.3	0.19
	Lag3	22.8	1.7	-5.6-9.1	0.64
	Lag4	29.2	1.4	-4.8-7.5	0.66
	Lag5	38.9	1.1	-8.5-10.7	0.82
	Lag6	34.6	0.3	-7.0-7.6	0.94
R ₅ -R ₂₀ , cmH ₂ O/(L/s)	Lag0	27.1	9.6	-7.8-27.0	0.28
	Lag1	30.3	15.8	0.4-31.1	0.045
	Lag2	32.0	11.3	-7.2-29.8	0.22
	Lag3	22.8	14.7	-4.6-34.1	0.13
	Lag4	29.2	10.4	-5.7-26.5	0.20
	Lag5	38.9	14.5	-10.2-39.2	0.25
	Lag6	34.6	5.3	-13.4-24.0	0.58
X ₅ , cmH ₂ O/(L/s)	Lag0	27.1	-5.8	-41.6-30.0	0.75
	Lag1	30.3	6.3	-25.1-37.8	0.69
	Lag2	32.0	30.9	-7.5-69.2	0.11
	Lag3	22.8	-4.8	-43.9-34.3	0.81
	Lag4	29.2	2.7	-36.2-30.8	0.87
	Lag5	38.9	11.3	-40.6-63.2	0.67
	Lag6	34.6	22.0	-18.3-62.4	0.28
Fres, Hz	Lag0	27.1	2.1	-7.6-11.8	0.67
	Lag1	30.3	0.096	-8.6-8.8	0.98
	Lag2	32.0	-8.4	-18.4-1.5	0.096
	Lag3	22.8	2.7	-8.1-13.5	0.62
	Lag4	29.2	-0.1	-9.1-8.9	0.98
	Lag5	38.9	-10.6	-24.0-2.9	0.12
	Lag6	34.6	-3.8	-14.1-6.5	0.47
FEV ₁ , L	Lag0	27.1	-0.6	-3.4-2.3	0.68
	Lag1	30.3	-1.5	-3.8-0.8	0.19
	Lag2	32.0	-0.2	-3.0-2.6	0.87
	Lag3	22.8	0.2	-2.7-3.1	0.91
	Lag4	29.2	-0.8	-3.2-1.8	0.55

	Lag5	38.9	-2.6	-6.4-1.4	0.20
	Lag6	34.6	-1.7	-4.7-1.4	0.28
FVC, L	Lag0	27.1	-1.3	-3.3-0.70	0.19
	Lag1	30.3	-1.4	-3.1-0.30	0.10
	Lag2	32.0	0.55	-1.4-2.5	0.57
	Lag3	22.8	-0.72	-2.8-1.4	0.50
	Lag4	29.2	-0.96	-2.7-0.8	0.28
	Lag5	38.9	-1.9	-4.7-0.90	0.18
	Lag6	34.6	-0.89	-3.0-1.3	0.42
PEF, L/s	Lag0	27.1	0.68	-3.1-4.5	0.72
	Lag1	30.3	-1.6	-4.7-1.6	0.32
	Lag2	32.0	-0.6	-4.3-3.1	0.74
	Lag3	22.8	1.5	-2.4-5.3	0.46
	Lag4	29.2	-0.39	-3.7-2.9	0.82
	Lag5	38.9	-0.66	-6.1-4.7	0.81
	Lag6	34.6	-2.6	-6.7-1.5	0.21
FEF ₂₅₋₇₅ , L/s	Lag0	27.1	-1.6	-7.5-4.6	0.60
	Lag1	30.3	-2.5	-7.3-2.7	0.34
	Lag2	32.0	-1.3	-7.1-4.9	0.67
	Lag3	22.8	0.3	-5.9-6.9	0.93
	Lag4	29.2	-1.1	-6.5-4.5	0.68
	Lag5	38.9	-4.5	-12.4-4.0	0.28
	Lag6	34.6	-4.0	-10.1-2.6	0.23
FEV ₁ /FVC	Lag0	27.1	1.1	-0.83-3.0	0.26
	Lag1	30.3	0.087	-1.5-1.7	0.92
	Lag2	32.0	-0.61	-2.6-1.4	0.54
	Lag3	22.8	1.4	-0.59-3.5	0.16
	Lag4	29.2	0.42	-1.4-2.	0.65
	Lag5	38.9	-0.18	-2.9-2.6	0.90
	Lag6	34.6	-0.65	-2.7-1.5	0.55
FeNO, ppb	Lag0	27.1	12.1	1.3-24.2	0.028
	Lag1	30.3	9.6	0.74-19.3	0.033
	Lag2	32.0	6.5	-4.0-18.2	0.23
	Lag3	22.8	20.6	8.9-33.5	0.0004
	Lag4	29.2	18.2	8.4-28.9	0.0002
	Lag5	38.9	25.3	9.5-43.4	0.001
	Lag6	34.6	8.8	-2.1-21.0	0.12

Table E6. Estimated means and 95% confident intervals for change in biomarkers (%) with one IQR increase in 24-hour average PM_{2.5} in zero to six days prior to health outcome measurement (lag 0-6) in participants with blood eosinophil counts ≤ 450/μL (For Figure 20).

Health Outcomes	PM _{2.5} Exposures	IQR, μg/m ³	Mean Changes (%)	95% CI	p-value
Z ₅ , cmH ₂ O/(L/s)	Lag0	24.2	6.2	-0.57-12.9	0.072
	Lag1	31.0	3.9	-2.2-9.9	0.21
	Lag2	33.2	4.9	-2.0-11.8	0.16
	Lag3	23.8	8.4	0.89-15.9	0.029
	Lag4	29.6	9.9	2.6-17.3	0.0089
	Lag5	38.9	9.3	0.52-18.2	0.038
	Lag6	36.7	3.7	-3.7-11.1	0.32
R ₅ , cmH ₂ O/(L/s)	Lag0	24.2	10.4	1.4-19.4	0.024
	Lag1	31.0	8.6	0.7-16.5	0.033
	Lag2	33.2	10.0	0.78-19.2	0.034
	Lag3	23.8	12.5	2.0-23.1	0.021
	Lag4	29.6	9.6	-0.8-20.0	0.071
	Lag5	38.9	11.0	-0.98-23.0	0.071
	Lag6	36.7	4.8	-4.9-15.6	0.33
R ₂₀ , cmH ₂ O/(L/s)	Lag0	24.2	5.9	-2.9-14.6	0.19
	Lag1	31.0	2.6	-4.8-9.9	0.49
	Lag2	33.2	6.9	-1.9-15.7	0.12
	Lag3	23.8	5.7	-4.4-15.9	0.26
	Lag4	29.6	7.5	-2.3-17.3	0.13
	Lag5	38.9	4.7	-6.8-16.1	0.42
	Lag6	36.7	1.9	-7.3-11.1	0.68
R ₅ -R ₂₀ , cmH ₂ O/(L/s)	Lag0	24.2	18.9	-3.4-41.3	0.096
	Lag1	31.0	20.6	1.2-39.9	0.037
	Lag2	33.2	15.9	-8.0-39.7	0.19
	Lag3	23.8	26.0	-0.26-52.3	0.052
	Lag4	29.6	14.0	-11.9-39.9	0.29
	Lag5	38.9	21.0	-8.5-50.4	0.16
	Lag6	36.7	7.4	-16.5-31.2	0.54
X ₅ , cmH ₂ O/(L/s)	Lag0	24.2	-21.5	-74.9-31.9	0.42
	Lag1	31.0	-4.8	-51.0-51.3	0.84
	Lag2	33.2	47.2	-6.9-101	0.085
	Lag3	23.8	-33.1	92.5-26.3	0.27
	Lag4	29.6	-27.2	84.329.8	0.34
	Lag5	38.9	-13.2	-84.3-58.0	0.71
	Lag6	36.7	19.7	-37.5-76.8	0.49
Fres, Hz	Lag0	24.2	7.5	-5.2-20.2	0.24
	Lag1	31.0	0.74	-10.3-11.8	0.89
	Lag2	33.2	-9.7	-22.8-3.4	0.15
	Lag3	23.8	9.0	-6.3-24.3	0.24
	Lag4	29.6	8.7	-5.9-23.4	0.24
	Lag5	38.9	-4.7	-21.5-12.2	0.58
	Lag6	36.7	-8.3	-21.9-5.3	0.23
FEV ₁ , L	Lag0	24.2	-0.67	-3.7-2.5	0.67
	Lag1	31.0	-1.4	-4.2-1.4	0.30

	Lag2	33.2	-0.16	-3.4-3.2	0.92
	Lag3	23.8	-0.72	-4.3-3.0	0.69
	Lag4	29.6	-2.5	-5.8-0.93	0.15
	Lag5	38.9	-5.2	-9.1 to -1.1	0.013
	Lag6	36.7	-2.3	-5.9-1.4	0.21
FVC, L	Lag0	24.2	-1.1	-3.2-1.1	0.32
	Lag1	31.0	-0.98	-2.8-0.93	0.30
	Lag2	33.2	0.65	-1.5-2.9	0.56
	Lag3	23.8	-0.92	-3.3-1.5	0.45
	Lag4	29.6	-1.6	-3.9-0.73	0.17
	Lag5	38.9	-3.0	-5.8 to -0.078	0.045
	Lag6	36.7	-0.77	-3.3-1.8	0.54
PEF, L/s	Lag0	24.2	-0.61	-5.3-4.1	0.80
	Lag1	31.0	-2.8	-6.8-1.2	0.17
	Lag2	33.2	-2.9	-7.3-1.6	0.20
	Lag3	23.8	-0.25	-5.4-4.9	0.92
	Lag4	29.6	-1.6	-6.7-3.4	0.52
	Lag5	38.9	-4.5	-10.5-1.6	0.14
	Lag6	36.7	-5.2	-10.4 to -0.014	0.049
FEF ₂₅₋₇₅ , L/s	Lag0	24.2	-1.7	-8.0-5.1	0.61
	Lag1	31.0	-2.9	-8.5-3.0	0.32
	Lag2	33.2	-2.3	-8.6-4.5	0.49
	Lag3	23.8	-2.7	-10.0-5.2	0.48
	Lag4	29.6	-5.3	-12.1-2.0	0.15
	Lag5	38.9	-8.4	-16.2-0.1	0.053
	Lag6	36.7	-6.0	-12.2-1.2	0.097
FEV ₁ /FVC	Lag0	24.2	0.65	-1.5-2.9	0.55
	Lag1	31.0	-0.41	-2.4-1.6	0.67
	Lag2	33.2	-0.83	-3.0-1.4	0.46
	Lag3	23.8	0.91	-1.7-3.6	0.49
	Lag4	29.6	-0.64	-3.1-1.9	0.60
	Lag5	38.9	-2.4	-5.0-0.41	0.09
	Lag6	36.7	-1.6	-3.9-0.79	0.18
FeNO, ppb	Lag0	24.2	21.7	8.4-36.7	0.001
	Lag1	31.0	10.8	-0.3-23.2	0.057
	Lag2	33.2	6.6	-6.5-21.5	0.34
	Lag3	23.8	30.8	14.8-48.9	0.00013
	Lag4	29.6	25.4	9.8-43.1	0.0012
	Lag5	38.9	28.8	10.6-49.9	0.0016
	Lag6	36.7	14.3	-0.25-31.0	0.054

Appendix F

This chapter is adapted with permission from He, L.; Cui, X.; Li, Z.; Teng, Y.; Barkjohn, K. K.; Norris, C.; Fang, L.; Lin, L.; Wang, Q.; Zhou, X.; Hong, J.; Li, F.; Zhang, Y.; Schauer, J. J.; Black, M.; Bergin, M.; Zhang, J., Malondialdehyde in Nasal Fluid: A Biomarker for Monitoring Asthma Control in Relation to Air Pollution Exposure. *Environmental Science and Technology*. 2020 (Publisher: American Chemical Society).

F1. Air purifier operation schedule

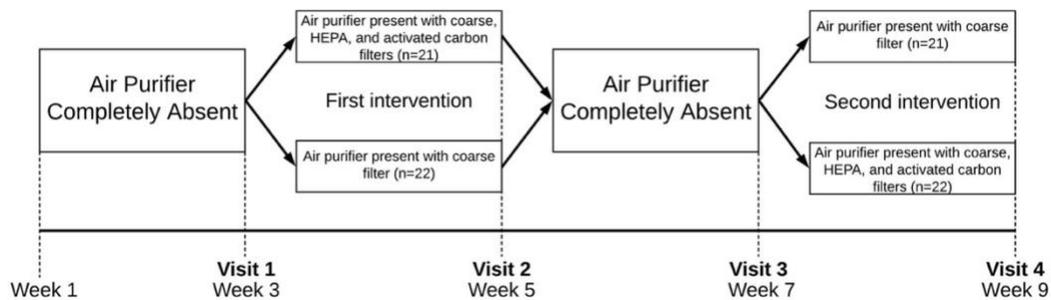


Figure F1. Operation schedule of air purifier in relation to the clinic visits.

F2. Statistical analysis

F2.1 Exposure-response relationships

Linear mixed-effect regression (LMER) models were constructed to determine the exposure-response association of personal air pollutant exposure with biomarkers of oxidative stress and C-ACT scores following *Formula F1*. The co-pollutant models were used as described in *Formula F2*. The dependent variable in the model was each of the biomarkers, and the independent variable was the personal air pollutant exposures

measured zero to six days prior to the clinical visits. Biomarkers and pollutant exposure were natural logarithm-transformed due to skewed distributions. We controlled for the fixed-effects covariates including ambient temperature, ambient relative humidity, urinary aMT6s (only for the 2 urinary biomarkers), respiratory tract infection status, inhaled corticosteroids usage, asthma exacerbation status, baseline eosinophil count, sleep duration, travel status (whether or not the participant was out of city during the two weeks prior to each clinical visit), sex, and age. We controlled for subject ID and the day of the week for clinical visit as random-effect variables. To investigate whether asthma exacerbation status, the inhaled corticosteroids usage, eosinophil inflammation status, or sex would modify the relationship between pollutant exposure and nasal MDA, the interaction term between pollutant exposure and these four factors was added as shown in *Formula F3*.

From the model output, we calculated percent change (and 95% confidence interval) of the biomarkers associated with an IQR increase in personal pollutant exposure.

Formula 1

$$\begin{aligned}
 Y_{ij} \sim & \beta_0 + \beta_1 Pol_{ij} + \beta_2 RH_{ij} + \beta_3 Temp_{ij} + \beta_4 Sex_i + \beta_5 Age_i + \beta_6 EOS_i + \beta_7 Fever_{ij} \\
 & + \beta_8 ICS_{ij} + \beta_9 Sleep_{ij} + \beta_{10} aMT6s_{ij} + \beta_{11} Travel_{ij} + \beta_{12} Flare_{ij} \\
 & + W_i + P_i + \epsilon
 \end{aligned}$$

Formula 2

$$Y_{ij} \sim \beta_0 + \beta_1 Pol_{ij} + \beta_2 RH_{ij} + \beta_3 Temp_{ij} + \beta_4 Sex_i + \beta_5 Age_i + \beta_6 EOS_i + \beta_7 Fever_{ij} + \beta_8 ICS_{ij} \\ + \beta_9 Sleep_{ij} + \beta_{10} aMT6s_{ij} + \beta_{11} Travel_{ij} + \beta_{12} Flare_{ij} + \beta_{13} Second_Pol_{ij} \\ + W_i + P_i + \epsilon$$

Formula 3

$$Y_{ij} \sim \beta_0 + \beta_1 Pol_{ij} + \beta_2 RH_{ij} + \beta_3 Temp_{ij} + \beta_4 Sex_i + \beta_5 Age_i + \beta_6 EOS_i + \beta_7 Fever_{ij} + \beta_8 ICS_{ij} \\ + \beta_9 Sleep_{ij} + \beta_{10} aMT6s_{ij} + \beta_{11} Travel_{ij} + \beta_{12} Flare_{ij} + \beta_{13} Pol_{ij} \\ * Modifier_{ij} + W_i + P_i + \epsilon$$

F2.2 Relationships of biomarkers of oxidative stress and inflammation in the upper respiratory tract with respiratory symptoms.

LMER models were used to examine the associations of nasal fluid and salivary biomarkers with the scores of C-ACT questions following *Formula 4*. In these models, the scores of each of C-ACT question were the dependent variables, and the concentration of the biomarkers of oxidative stress and airway inflammation would be the independent variables along with the covariate structure described in the first model. From the model output, we calculated percent change (95% confidence interval) in the C-ACT scores associated with an IQR increase in the biomarkers.

Formula 4

$$C - ACT_{ij} \sim \beta_0 + \beta_1 NF \text{ or Salivary Biomarkrs}_{ij} + \beta_2 RH_2w_{ij} + \beta_3 Temp_2w_{ij} + \beta_4 Sex_i \\ + \beta_5 Age_i + \beta_6 EOS_i + \beta_7 Fever_{ij} + \beta_8 ICS_{ij} + \beta_9 Sleep_{ij} + \beta_{10} aMT6s_{ij} \\ + \beta_{11} Travel_{ij} + \beta_{12} Flare_{ij} + W_i + P_i + \epsilon$$

Codebook

i: participant id number ($i = 1, 2, \dots, 43$)

j: sample number ($j = 1, 2, 3, 4$)

Y_{ij}: concentration of biomarkers of oxidative stress and inflammation, including urinary MDA (ng/mg creatinine), urinary 8-OHdG (ng/mg creatinine), nasal MDA (ng/mL).

Pol_{ij}: 24-hour average personal PM_{2.5} exposure and the maximum 8-hour average personal O₃ exposure measured zero to six days prior to the clinical visit (nature logarithm transformed) (unit: PM_{2.5}: $\mu\text{g}/\text{m}^3$ and O₃: ppb)

Second_Pol_{ij}: co-pollutant O₃ exposure (unit: ppb)

RH_{ij}: 24-hour average ambient relative humidity measured zero to six days prior to health indicators measurement (unit: %)

RH_2w_{ij}: ambient relative humidity averaged over 2-week prior to health indicators measurement (unit: %)

Temp_{ij}: 24-hour average ambient temperature measured zero to six days prior to health indicators measurement, a natural spline with degrees of freedom equals 3 was applied (unit: °C)

Temp_2w_{ij}: ambient temperature averaged over 2-week prior to health indicators measurement (unit: °C)

Age_i: subjects' age (unit: year)

Sex_i: subjects' sex (0=male, 1=female)

EOS_i: baseline eosinophil number (unit: $/\mu\text{L}$)

Fever_{ij}: upper respiratory tract infection like symptoms status during 2 weeks prior to the clinical visit (0=non-respiratory infection symptoms, 1=respiratory infection symptoms)

ICS_{ij}: inhaled corticosteroids usage status during 2 weeks prior to the clinical visit (0=not used, 1=used)

Sell_{ij}: sleep duration for the night prior to the clinical visit (unit: hour)

Travel_{ij}: status of traveling during 2-weeks prior to each of the clinical visit (0=no-travel, 1=travel)

aMT6s_{ij}: concentration of aMT6s (unit: ng/mg creatinine)

C-ACT_{ij}: scores of the first four questions of C-ACT (value=0, 1, 2, 3)

Flare_{ij}: the status of asthma exacerbation during 2 weeks prior to the clinical visit (0=no exacerbation, 1=exacerbation)

Modifier_{ij}: asthma exacerbation status, the inhaled corticosteroids usage, eosinophil inflammation status, or sex

W_i: random intercept for the day of the week for clinical visit

P_i: individual-specific random intercept

ϵ : residual

F3. I/O ratios

Table F1. I/O ratio for each microenvironment.

	I/O Ratio	
	O ₃	PM _{2.5}
Car/Taxi	0.4	0.8
Subway	0.35	0.8
Walk/ Bike/outdoor exercise	1	1
Other indoor environment outside home (window closed)	0.35	0.8
Other indoor environment outside home (window closed)	0.85	1
Bedroom (window closed, during the filtration period)	Mean Home specific I/O ratio \pm SD (0.35 \pm 0.42)	Mean Home specific I/O ratio \pm SD (0.44 \pm 0.60)
Bedroom (window closed, during the no filtration period)	0.35	0.8
Bedroom (window opened)	0.85	1

F4. Detailed model results

Table F2. The model results of Figure 21.

Biomarker		PM _{2.5}				O ₃			
		Effect size	CI (lower)	CI (upper)	P value	Effect size	CI (lower)	CI (upper)	P value
Nasal MDA	lag 0	38.6	14.3	68.1	0.00	20.3	-0.2	45.2	0.05
Nasal MDA	lag 1	39.2	16.4	66.6	0.00	25.6	-1.0	59.5	0.06
Nasal MDA	lag 2	51.0	19.1	91.5	0.00	22.1	0.9	47.9	0.04
Nasal MDA	lag 3	54.9	20.6	99.1	0.00	69.4	40.3	104.6	0.00
Nasal MDA	lag 4	49.8	20.3	86.5	0.00	25.1	4.6	49.7	0.01
Nasal MDA	lag 5	42.3	8.1	87.5	0.01	-0.4	-21.4	26.3	0.98
Nasal MDA	lag 6	22.5	-1.0	51.4	0.06	2.1	-18.7	28.1	0.86
FeNO	lag 0	4.0	-4.7	13.4	0.38	-6.0	-14.5	3.4	0.20
FeNO	lag 1	2.6	-4.8	10.6	0.50	-0.8	-10.4	9.8	0.88
FeNO	lag 2	1.0	-8.2	11.1	0.84	-2.2	-10.4	6.7	0.61
FeNO	lag 3	8.6	-1.1	19.2	0.08	3.4	-5.4	13.1	0.46
FeNO	lag 4	10.2	1.2	20.0	0.03	2.1	-5.0	9.9	0.57
FeNO	lag 5	9.4	-1.4	21.4	0.09	6.0	-4.4	17.5	0.27
FeNO	lag 6	6.0	-2.8	15.5	0.19	1.5	-9.0	13.2	0.79
Urinary MDA	lag 0	2.2	-9.8	15.7	0.73	0.5	-12.4	15.4	0.94
Urinary MDA	lag 1	10.2	-1.2	22.8	0.08	-6.0	-19.0	9.1	0.41
Urinary MDA	lag 2	12.5	-1.9	29.0	0.09	5.9	-6.9	20.4	0.38
Urinary MDA	lag 3	-2.0	-14.4	12.2	0.77	2.3	-9.5	15.6	0.71
Urinary MDA	lag 4	-1.3	-12.5	11.3	0.83	3.0	-6.5	13.5	0.55
Urinary MDA	lag 5	-3.5	-17.0	12.3	0.64	-12.2	-24.2	1.8	0.08
Urinary MDA	lag 6	0.2	-11.2	12.9	0.98	-4.7	-17.7	10.4	0.52
Urinary 8-OHdG	lag 0	-8.8	-43.0	45.9	0.70	-22.8	-50.8	21.1	0.26
Urinary 8-OHdG	lag 1	-6.1	-38.7	43.9	0.77	-17.6	-52.8	43.7	0.49
Urinary 8-OHdG	lag 2	-19.1	-53.1	39.5	0.44	-0.6	-36.8	56.3	0.98
Urinary 8-OHdG	lag 3	-12.1	-49.0	51.4	0.64	-9.5	-42.0	41.4	0.66
Urinary 8-OHdG	lag 4	-12.6	-45.7	40.8	0.58	25.7	-13.8	83.4	0.23
Urinary 8-OHdG	lag 5	-8.1	-49.1	65.8	0.78	23.5	-27.3	109.8	0.43
Urinary 8-OHdG	lag 6	-2.7	-39.2	55.9	0.91	40.7	-15.9	135.3	0.19

Table F3. The model results of Figure 22.

Biomarker		PM _{2.5}				O ₃			
		Effect size	CI (lower)	CI (upper)	P value	Effect size	CI (lower)	CI (upper)	P value
Asthma control	lag 0	-3.5	-8.5	1.4	0.16	4.0	-1.0	8.9	0.12
Asthma control	lag 1	-4.4	-8.9	0.1	0.05	5.0	-0.9	11.0	0.10
Asthma control	lag 2	-7.6	-12.8	-2.5	0.00	5.7	0.5	10.9	0.03
Asthma control	lag 3	-3.1	-8.4	2.1	0.24	-1.4	-6.0	3.2	0.55
Asthma control	lag 4	-6.6	-11.3	-1.8	0.01	-1.8	-5.5	1.9	0.34
Asthma control	lag 5	-7.7	-13.5	-1.8	0.01	-3.4	-9.4	2.5	0.25
Asthma control	lag 6	-5.3	-10.1	-0.6	0.03	-0.6	-6.4	5.3	0.85
Limitation of physical activities	lag 0	-2.9	-9.5	3.8	0.39	5.7	-0.8	12.2	0.09
Limitation of physical activities	lag 1	-7.3	-13.2	-1.4	0.02	-2.4	-10.5	5.7	0.55
Limitation of physical activities	lag 2	-3.0	-10.2	4.3	0.42	-4.2	-11.4	3.0	0.25
Limitation of physical activities	lag 3	-4.7	-12.1	2.6	0.20	-1.2	-7.5	5.2	0.72
Limitation of physical activities	lag 4	-2.8	-9.7	4.1	0.42	-1.6	-6.8	3.6	0.55
Limitation of physical activities	lag 5	0.8	-7.0	8.7	0.83	-3.8	-11.6	3.9	0.33
Limitation of physical activities	lag 6	2.2	-4.0	8.4	0.49	-2.0	-9.5	5.6	0.61
Coughing	lag 0	0.0	-8.0	8.1	0.99	7.6	0.0	15.2	0.05
Coughing	lag 1	-7.3	-14.4	-0.1	0.05	-0.7	-10.3	8.9	0.88
Coughing	lag 2	-2.8	-11.7	6.1	0.53	-6.5	-15.0	2.0	0.13
Coughing	lag 3	-3.2	-11.9	5.5	0.47	-0.6	-7.9	6.7	0.87
Coughing	lag 4	-8.0	-16.2	0.1	0.05	-2.8	-9.0	3.3	0.36
Coughing	lag 5	-4.1	-13.4	5.2	0.39	-3.6	-12.6	5.4	0.43
Coughing	lag 6	1.4	-6.1	9.0	0.71	2.5	-6.4	11.3	0.58
Waking up at night	lag 0	-0.4	-3.4	2.7	0.82	-0.2	-3.2	2.8	0.90
Waking up at night	lag 1	-1.5	-4.2	1.2	0.28	-3.9	-7.4	-0.3	0.03
Waking up at night	lag 2	-2.4	-5.8	1.0	0.17	-3.3	-6.5	0.0	0.05
Waking up at night	lag 3	-0.9	-4.3	2.6	0.62	-3.1	-5.9	-0.2	0.04
Waking up at night	lag 4	-2.5	-5.7	0.7	0.12	-2.9	-5.2	-0.5	0.02
Waking up at night	lag 5	-1.0	-4.7	2.8	0.60	-3.8	-7.4	-0.2	0.04
Waking up at night	lag 6	-1.0	-4.0	2.0	0.53	-4.1	-7.6	-0.7	0.02
Total C-ACT score (Child)	lag 0	-1.3	-5.3	2.6	0.50	4.0	0.3	7.8	0.03
Total C-ACT score (Child)	lag 1	-4.7	-8.2	-1.2	0.01	-0.5	-5.2	4.2	0.84
Total C-ACT score (Child)	lag 2	-3.7	-8.0	0.5	0.09	-2.1	-6.3	2.1	0.33
Total C-ACT score (Child)	lag 3	-2.9	-7.2	1.4	0.18	-1.6	-5.2	2.1	0.39
Total C-ACT score (Child)	lag 4	-4.6	-8.6	-0.7	0.02	-2.1	-5.1	0.9	0.17
Total C-ACT score (Child)	lag 5	-2.9	-7.4	1.7	0.21	-3.8	-8.2	0.6	0.09
Total C-ACT score (Child)	lag 6	-0.7	-4.4	3.1	0.73	-1.2	-5.6	3.1	0.58
Daytime asthma symptoms	lag 0	-2.9	-6.5	0.6	0.11	-0.3	-3.7	3.2	0.87
Daytime asthma symptoms	lag 1	-1.2	-4.4	2.0	0.46	-4.1	-8.3	0.1	0.06
Daytime asthma symptoms	lag 2	0.5	-3.2	4.3	0.77	-1.9	-5.5	1.8	0.32
Daytime asthma symptoms	lag 3	-3.1	-6.9	0.7	0.10	-0.6	-3.8	2.5	0.70
Daytime asthma symptoms	lag 4	-2.1	-5.6	1.4	0.24	-1.4	-4.0	1.3	0.31
Daytime asthma symptoms	lag 5	-4.6	-8.6	-0.5	0.03	-1.4	-5.5	2.6	0.48
Daytime asthma symptoms	lag 6	-0.8	-4.3	2.7	0.64	-0.9	-4.9	3.2	0.67
Wheezing	lag 0	-0.9	-3.5	1.7	0.48	-0.6	-3.2	1.9	0.62
Wheezing	lag 1	-2.1	-4.4	0.2	0.07	-0.9	-4.0	2.2	0.58
Wheezing	lag 2	-1.4	-4.2	1.4	0.32	0.0	-2.7	2.8	0.98
Wheezing	lag 3	-1.7	-4.4	1.0	0.21	-0.6	-2.9	1.8	0.63
Wheezing	lag 4	-1.5	-4.1	1.1	0.26	-1.3	-3.2	0.7	0.20
Wheezing	lag 5	-4.6	-7.5	-1.6	0.00	-1.2	-4.2	1.8	0.43
Wheezing	lag 6	-2.4	-4.8	0.1	0.06	-0.6	-3.6	2.4	0.68
Waking up at night	lag 0	-0.7	-2.7	1.2	0.46	1.4	-0.3	3.2	0.11
Waking up at night	lag 1	-0.8	-2.5	1.0	0.39	0.5	-1.9	2.8	0.69
Waking up at night	lag 2	0.8	-1.5	3.0	0.50	-0.8	-2.8	1.2	0.41
Waking up at night	lag 3	-0.6	-2.9	1.6	0.58	0.2	-1.6	1.9	0.85
Waking up at night	lag 4	-1.8	-3.8	0.2	0.08	-0.4	-1.9	1.1	0.61
Waking up at night	lag 5	-1.9	-4.2	0.4	0.11	-0.4	-2.5	1.7	0.71
Waking up at night	lag 6	-0.4	-2.4	1.5	0.68	0.2	-1.9	2.4	0.83
Total C-ACT score (Caregiver)	lag 0	-1.6	-3.7	0.6	0.15	0.2	-1.9	2.3	0.86
Total C-ACT score (Caregiver)	lag 1	-1.4	-3.3	0.5	0.16	-1.5	-4.1	1.0	0.24
Total C-ACT score (Caregiver)	lag 2	-0.1	-2.3	2.2	0.95	-1.0	-3.2	1.2	0.39
Total C-ACT score (Caregiver)	lag 3	-1.9	-4.1	0.3	0.10	-0.5	-2.4	1.3	0.57
Total C-ACT score (Caregiver)	lag 4	-1.7	-3.8	0.4	0.11	-1.1	-2.7	0.5	0.19
Total C-ACT score (Caregiver)	lag 5	-3.7	-6.0	-1.3	0.00	-1.1	-3.5	1.3	0.38
Total C-ACT score (Caregiver)	lag 6	-1.3	-3.3	0.8	0.22	-0.4	-2.8	2.0	0.73

Table F4. The model results of Figure 23.

C-ACT	Biomarker	Effect size	CI (lower)	CI (upper)	P value
Asthma control	Nasal MDA	-3.7	-9.3	1.9	0.20
Asthma control	FeNO	0.4	-7.3	8.1	0.91
Asthma control	Urinary MDA	3.3	-1.1	7.7	0.14
Asthma control	Urinary 8-OHdG	0.7	-3.6	5.1	0.74
Limitation of physical activities	Nasal MDA	-8.8	-16.3	-1.2	0.02
Limitation of physical activities	FeNO	9.9	-0.4	20.1	0.06
Limitation of physical activities	Urinary MDA	-4.6	-10.4	1.2	0.12
Limitation of physical activities	Urinary 8-OHdG	-2.0	-7.8	3.9	0.51
Coughing	Nasal MDA	-12.7	-21.5	-3.9	0.01
Coughing	FeNO	8.7	-3.3	20.6	0.15
Coughing	Urinary MDA	3.5	-3.6	10.5	0.33
Coughing	Urinary 8-OHdG	5.1	-1.9	12.1	0.15
Waking up at night	Nasal MDA	-2.8	-6.5	0.8	0.12
Waking up at night	FeNO	-0.4	-5.2	4.4	0.87
Waking up at night	Urinary MDA	0.2	-2.6	2.9	0.90
Waking up at night	Urinary 8-OHdG	0.4	-2.5	3.2	0.80
Total C-ACT score	Nasal MDA	-6.7	-11.0	-2.4	0.00
Total C-ACT score	FeNO	4.6	-1.3	10.5	0.13
Total C-ACT score	Urinary MDA	0.6	-2.9	4.1	0.75
Total C-ACT score	Urinary 8-OHdG	1.0	-2.5	4.5	0.58
Daytime asthma symptoms	Nasal MDA	-1.4	-5.4	2.6	0.49
Daytime asthma symptoms	FeNO	1.2	-4.0	6.5	0.64
Daytime asthma symptoms	Urinary MDA	-1.3	-4.4	1.7	0.39
Daytime asthma symptoms	Urinary 8-OHdG	-0.5	-3.6	2.6	0.74
Wheezing	Nasal MDA	-1.7	-4.7	1.3	0.26
Wheezing	FeNO	2.9	-1.2	6.9	0.16
Wheezing	Urinary MDA	1.2	-1.0	3.5	0.29
Wheezing	Urinary 8-OHdG	-0.2	-2.5	2.1	0.86
Wakeing up at night	Nasal MDA	0.6	-1.7	2.9	0.59
Wakeing up at night	FeNO	1.2	-1.5	3.9	0.38
Wakeing up at night	Urinary MDA	0.1	-1.3	1.6	0.89
Wakeing up at night	Urinary 8-OHdG	0.2	-1.6	1.9	0.85
Total C-ACT score	Nasal MDA	-0.8	-3.2	1.6	0.50
Total C-ACT score	FeNO	1.7	-1.5	4.8	0.29
Total C-ACT score	Urinary MDA	0.0	-1.7	1.7	0.99
Total C-ACT score	Urinary 8-OHdG	-0.2	-2.0	1.7	0.87

F5. Correlations among pollutant exposures

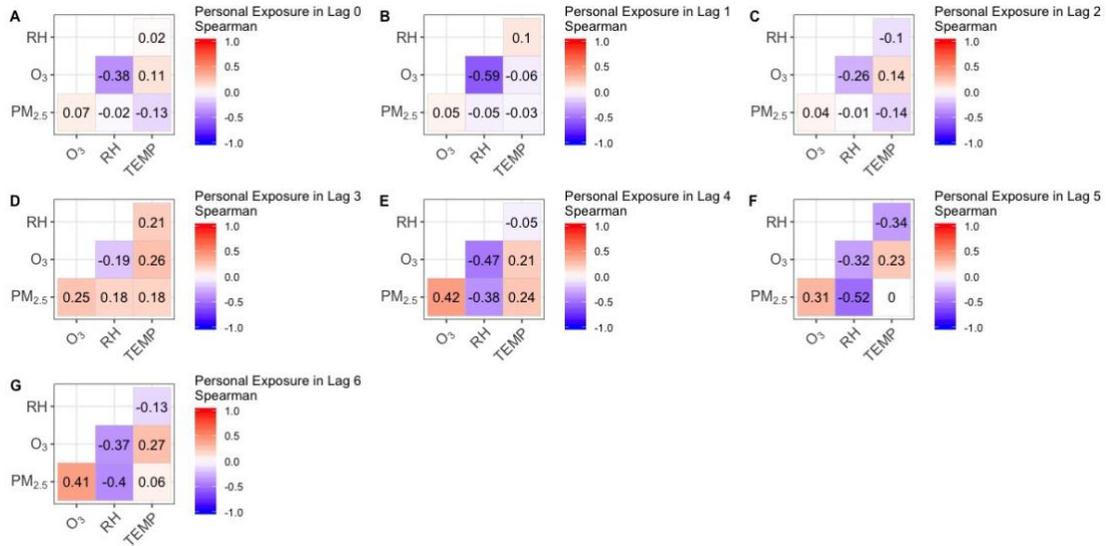


Figure F2. The correlations among air pollutant exposures, temperature, and relative humidity. RH: ambient relative humidity, TEMP: ambient temperature

F6. Sensitivity analyses

F6.1 Co-pollutant models

Co-pollutant models, following *Formula F2*, were developed to examine whether the exposure-response relationship obtained in the single-pollutant models can be retained after controlling for the co-pollutant. The exposure-response relationship between pollutant exposures and biomarkers of oxidative stress and inflammation were not remarkably different with respect to the significance or effect size after controlling the co-pollutant (Figure F3). We only found that the relationships of nasal MDA with personal O₃ exposure measured in two days and four days prior changed from significant to non-significant. Similarly, there were no noticeable changes in the

relationships of pollutant exposure and C-ACT scores in co-pollutant models (Figure F4).

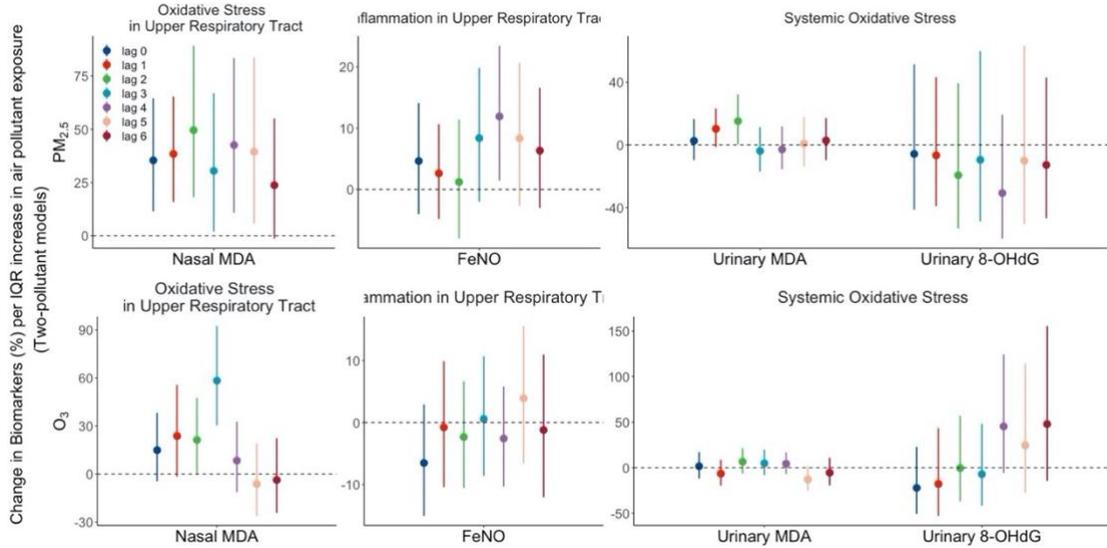


Figure F3. Estimated means and 95% confidence intervals for change in biomarkers (%) with one IQR increase in 24-hour average $PM_{2.5}$ and maximum daily 8-hour average O_3 personal exposure in zero to six days prior to clinic visits (lag 0-6). Co-pollutant exposure was adjusted in the models.

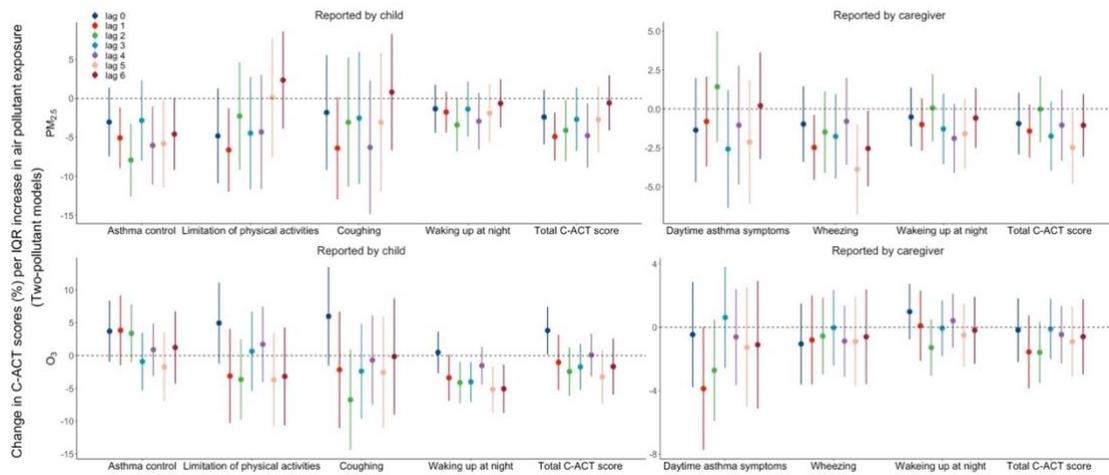


Figure F4. Estimated means and 95% confidence intervals for change in C-ACT outcomes (%) with one IQR increase in 24-hour average PM_{2.5} and maximum daily 8-hour average O₃ personal exposure in zero to six days prior to clinic visits (lag 0-6). Co-pollutant exposure was adjusted in the models.

F6.2 Excluding measurements of participants who suffered asthma exacerbation: removed 7 measurements from 6 participants.

Sensitivity analyses were conducted for the associations shown in the main content in a dataset excluding measurements of participants who suffered asthma exacerbation during the two weeks prior to the clinical visits following *Formula F1, F2, F4.*

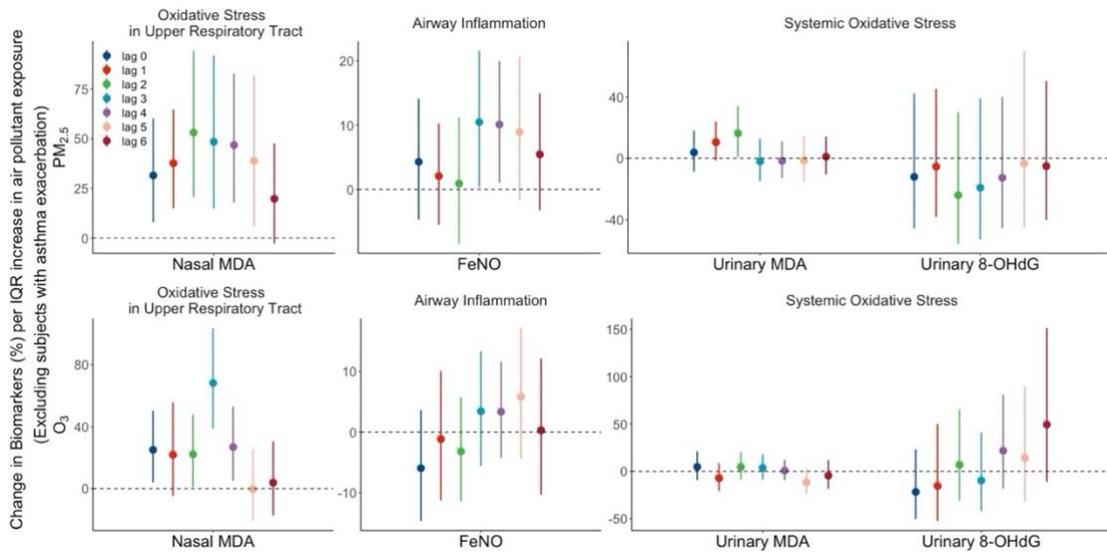


Figure F5. Estimated means and 95% confidence intervals for change in biomarkers (%) with one IQR increase in 24-hour average PM_{2.5} and maximum daily 8-hour average O₃ personal exposure in zero to six days prior to clinic visits (lag 0-6) excluding participants who suffered asthma exacerbation.

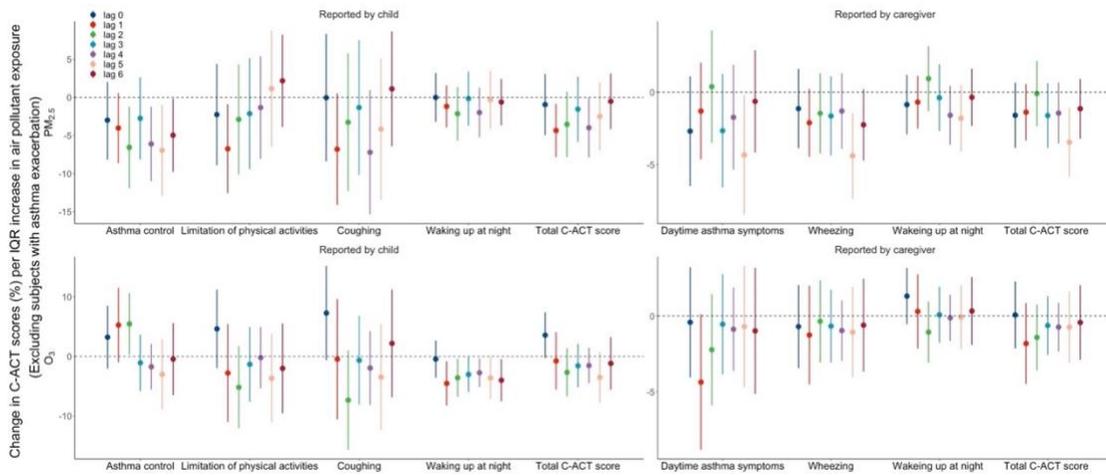


Figure F6. Estimated means and 95% confidence intervals for change in C-ACT outcomes (%) with one IQR increase in 24-hour average $PM_{2.5}$ and maximum daily 8-hour average O_3 personal exposure in zero to six days prior to clinic visits (lag 0-6) excluding participants who suffered asthma exacerbation.

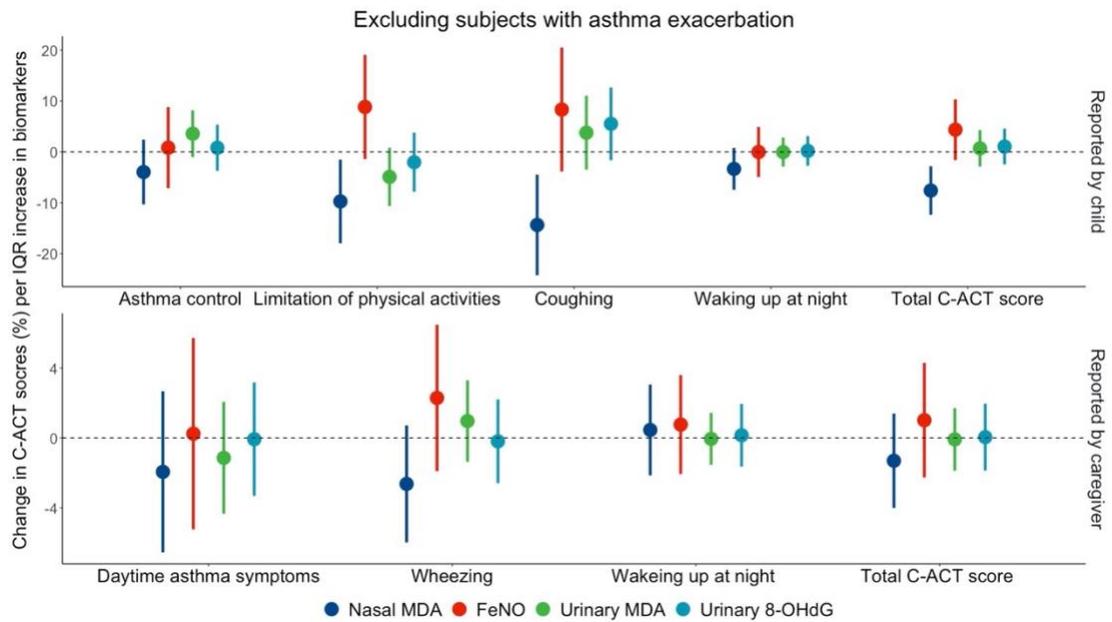


Figure F7. Estimated means and 95% confidence intervals for change in C-ACT scores (%) with one IQR increase in nasal MDA, FeNO, and urinary 8-OHdG excluding participants who suffered asthma exacerbation.

F6.3 Excluding measurements of participants who used inhaled corticosteroids: removed 31 measurements from 14 participants.

Sensitivity analyses were conducted for the associations shown in the main content in a dataset excluding measurements of participants who used inhaled corticosteroids during two weeks prior to the clinical visits following *Formula F1, F2, F4*.

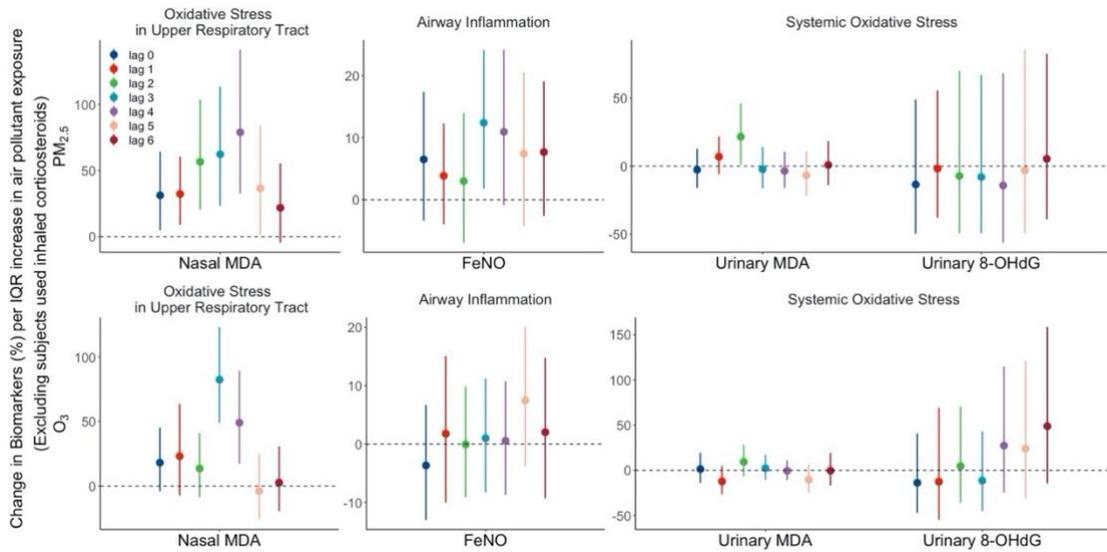


Figure F8. Estimated means and 95% confidence intervals for change in biomarkers (%) with one IQR increase in 24-hour average $PM_{2.5}$ and maximum daily 8-hour average O_3 personal exposure in zero to six days prior to clinic visits (lag 0-6), excluding participants who used inhaled corticosteroids.

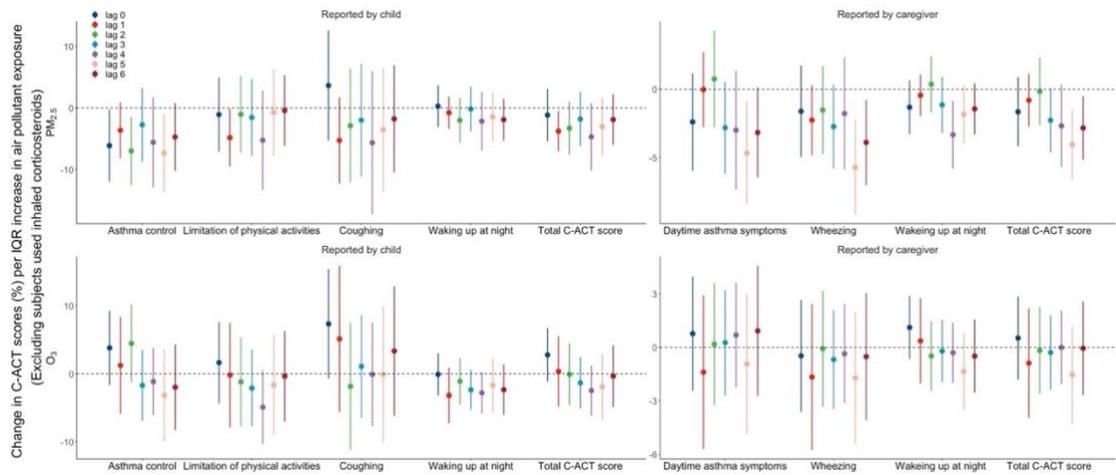


Figure F9. Estimated means and 95% confident intervals for change in C-ACT outcomes (%) with one IQR increase in 24-hour average PM_{2.5} and maximum daily 8-hour average O₃ personal exposure in zero to six days prior to clinic visits (lag 0-6), excluding participants who used inhaled corticosteroids.

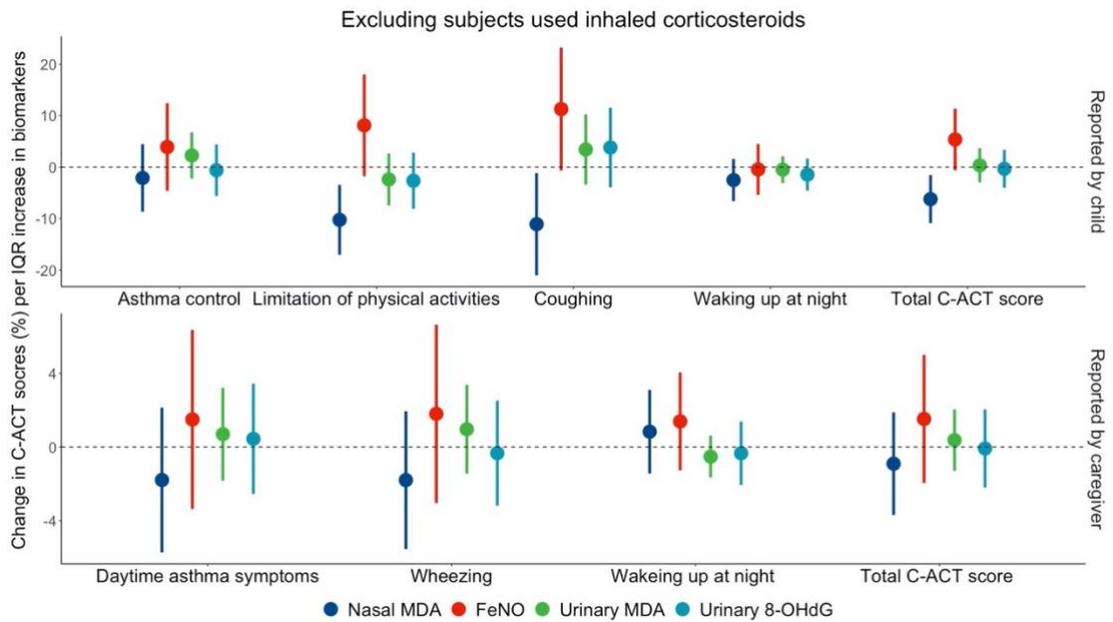


Figure F10. Estimated means and 95% confidence intervals for change in C-ACT scores (%) with one IQR increase in nasal MDA, FeNO, and urinary 8-OHdG excluding participants who used inhaled corticosteroids.

F6.4 Effects modification

To investigate whether the asthma exacerbation status or corticosteroids usage modify the relationship between air pollutant exposure and nasal MDA, we assessed the interaction between air pollution and these two factors in LMER models following

Formula F3. The model results of these interactions are shown in Table F4.

Table F4. The interactions of pollutant exposure with asthma exacerbation status, corticosteroid usage, eosinophil inflammation status, or sex.

	PM _{2.5} × asthma exacerbation interaction		PM _{2.5} × corticosteroids interaction		PM _{2.5} × eosinophil inflammation		PM _{2.5} × sex	
	β	p-value	β	p-value	β	p-value	β	p-value
Lag0	1.37	0.05	0.67	0.10	-0.40	0.17	-1.44	0.62
Lag1	1.30	0.21	0.43	0.24	-0.32	0.23	-0.23	0.31
Lag2	-0.36	0.54	-0.11	0.78	-0.14	0.61	-0.07	0.77
Lag3	0.20	0.78	-0.53	0.21	-0.49	0.18	-0.19	0.60
Lag4	0.81	0.37	-0.45	0.08	-0.66	0.009	0.17	0.50
Lag5	0.34	0.56	0.16	0.67	-0.32	0.25	-0.11	0.66
Lag6	0.31	0.53	0.39	0.23	-0.27	0.27	0.13	0.57
	O ₃ × asthma exacerbation interaction		O ₃ × corticosteroids interaction		O ₃ × eosinophil inflammation		O ₃ × sex	
	β	p-value	β	p-value	β	p-value	β	p-value
Lag0	-0.94	0.30	0.84	0.08	0.37	0.34	-0.11	0.76
Lag1	1.0	0.35	0.80	0.11	0.63	0.15	0.07	0.86
Lag2	-0.99	0.56	0.98	0.12	0.20	0.71	-0.31	0.53
Lag3	3.8	0.08	-1.3	0.03	-0.68	0.17	0.53	0.23
Lag4	0.33	0.80	-0.83	0.05	-1.1	0.008	0.48	0.23
Lag5	0.90	0.42	0.36	0.49	-0.079	0.86	0.60	0.14
Lag6	2.2	0.08	0.27	0.52	-0.33	0.40	-0.19	0.60

Appendix G

G1. Air purifier operation schedule

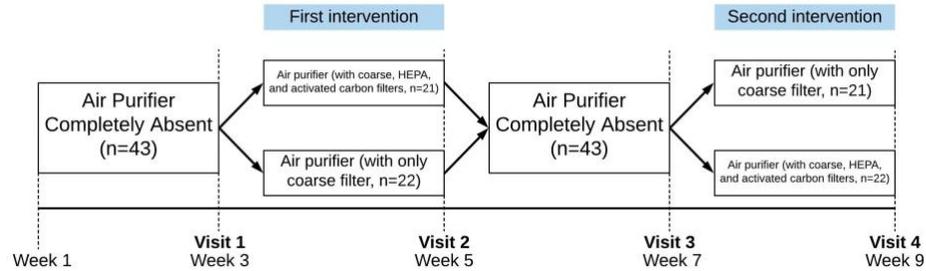


Figure G1. Schedule of air purifier operation.

G2. I/O ratios

Table G1. I/O ratio for each microenvironment.

	I/O Ratio	
	O ₃	PM _{2.5}
Other indoor environment outside home (window closed)	0.35	0.8
Other indoor environment outside home (window opened)	0.85	1
Bedroom (window closed, during the filtration period)	Mean Home specific I/O ratio ± SD (0.35 ± 0.42)	Mean Home specific I/O ratio ± SD (0.44 ± 0.60)
Bedroom (window closed, during the no filtration period)	0.35	0.8
Bedroom (window opened)	0.85	1
Car/Taxi	0.4	0.8
Subway	0.35	0.8
Walk/ Bike/outdoor exercise	1	1

G3. Statistical analysis

Linear mixed-effect regression (LMER) models were constructed to determine the exposure-response associations between urinary aMT6s and air pollution exposure following *Formula G1*. From the model output, we calculated percent change (and 95% confidence interval) of the urinary aMT6s concentration associated with an IQR increase in personal pollutant exposure.

Formula G1

$$aMT6s_{ij} \sim \beta_0 + \beta_1 Pol_{ij} + \beta_2 RH_{ij} + \beta_3 Temp_{ij} + \beta_4 RI_{ij} + \beta_5 ICS_{ij} + \beta_6 Sleep_{ij} + \beta_7 Travel_{ij} + \beta_8 Flare_{ij} + \beta_9 Age_i + \beta_{10} Sex_i + \beta_{11} Supplements_{ij} + \beta_{12} EOS_i + \beta_{13} Dust_i + P_i + Wday + \epsilon$$

Formula G2

$$aMT6s_{ij} \sim \beta_0 + \beta_1 Pol_{ij} + \beta_2 RH_{ij} + \beta_3 Temp_{ij} + \beta_4 RI_{ij} + \beta_5 ICS_{ij} + \beta_6 Sleep_{ij} + \beta_7 Travel_{ij} + \beta_8 Flare_{ij} + \beta_9 Age_i + \beta_{10} Sex_i + \beta_{11} Supplements_{ij} + \beta_{12} EOS_i + \beta_{13} Dust_i + \beta_{14} Second_Pol_{ij} + P_i + Wday + \epsilon$$

LMER models were also constructed to investigate the associations between urinary aMT6s and oxidative stress biomarkers (urinary MDA and 8-OHdG) following *Formula G3*.

Formula G3

$$aMT6s_{ij} \sim \beta_0 + \beta_1 Oxidative\ Stress_{ij} + \beta_2 RH_Lag0_{ij} + \beta_3 Temp_Lag0_{ij} + \beta_4 RI_{ij} + \beta_5 ICS_{ij} + \beta_6 Sleep_{ij} + \beta_7 Flare_{ij} + \beta_8 Age_i + \beta_9 Sex_i + \beta_{10} Supplements_{ij} + \beta_{11} EOS_i + \beta_{12} Dust_i + \beta_{13} PM_Lag0_{ij} + \beta_{14} Ozone_Lag0_{ij} + P_i + Wday + \epsilon$$

We used LMER models to investigate the associations between respiratory health outcomes and urinary aMT6s *Formula G4*.

Formula G4

$$Respiratory\ Health_{ij} \sim \beta_0 + \beta_1 aMT6s_{ij} + \beta_2 RH_Lag0_{ij} + \beta_3 Temp_Lag0_{ij} + \beta_4 RI_{ij} + \beta_5 ICS_{ij} + \beta_6 Sleep_{ij} + \beta_7 Flare_{ij} + \beta_8 Age_i + \beta_9 Sex_i + \beta_{10} Supplements_{ij} + \beta_{11} EOS_i + \beta_{12} Dust_i + \beta_{13} PM_Lag0_{ij} + \beta_{14} Ozone_Lag0_{ij} + P_i + Wday + \epsilon$$

Codebook

i: Participant id number. ($i = 1, 2, \dots, 43$)

j: Sample number ($j = 1, 2, 3, 4$)
aMT6s_{ij}: Concentration of aMT6s. Note: aMT6s was natural logarithm transformed
Pol_{ij}: Personal exposure to PM_{2.5} or O₃. Note: pollutant exposures were natural logarithm transformed
Second_Pol_{ij}: Copollutant exposure
Respiratory Health_{ij}: Concentration of respiratory health outcome measures, including airway mechanics ($Z_5, R_5, R_{20}, R_5-R_{20}, X_5, F_{res}$), lung function (FEV₁, FVC, PEF, FEF₂₅₋₇₅, FEV₁/FVC), and airway inflammation (FeNO). Note: FEV₁, FVC, FEF₂₅₋₇₅, FEV₁/FVC, FeNO were natural logarithm transformed
Oxidative Stress_{ij}: Concentration of MDA and 8-OHdG. Note: MDA and 8-OHdG were natural logarithm transformed
RH_{ij}: 24-hour average ambient relative humidity measured zero to five days prior to health indicators measurement, and a natural spline with degrees of freedom equals 4 was applied. (unit: %)

Temp_{ij}: 24-hour average ambient temperature measured zero to five days prior to health indicator measurement. (unit: °C)
RH_Lag0_{ij}: average ambient relative humidity measured in the preceding 24-hour, and a natural spline with degrees of freedom equals 4 was applied (unit: %)

Temp_Lag0_{ij}: average ambient temperature measured in the preceding 24-hour (unit: °C)
RI_{ij}: Upper respiratory tract infection like symptoms status during the two weeks prior to the clinical visit. (0=without respiratory infection symptoms, 1= with respiratory infection symptoms)
ICS_{ij}: Inhaled corticosteroids usage during the two weeks prior to the clinical visit. (0=not used, 1=used)
Sleep_{ij}: Sleep duration for the night prior to the clinical visit. (unit: hour)
Travel_{ij}: Status of traveling to outside Shanghai City during the two weeks prior to each clinical visit. (0=no travel, 1=travel)
Flare_{ij}: Status of asthma exacerbation during the two weeks prior to the clinical visit (0=no exacerbation, 1=exacerbation)
Sex_i: Sex (0=male, 1=female)
Age_i: Age
EOS_i: Baseline eosinophil number
Dust_i: Dust allergy status
Supplements_{ij}: Supplementation (i.e. vitamins and cod liver oil, and probiotics) usage status during the two weeks prior to the clinical visit (0=not used, 1=used)
PM_Lag0_{ij}: 24-hour average personal exposure to PM_{2.5} at Lag 0
Ozone_Lag0_{ij}: 24-hour average personal exposure to O₃ at Lag 0
Wday: day of the week for clinical visit
P_i: Individual-specific random intercept
ε: Residual

G4. Correlations among pollutant exposures

Table G2. The Pearson correlations among PM_{2.5} exposures, O₃ exposures, temperature, and relative humidity.

Lag 0			
	O ₃	Relative Humidity	Temperature
PM _{2.5}	0.07	-0.02	-0.13
O ₃		-0.38	0.11
Relative Humidity			0.02
Lag 1			
	O ₃	Relative Humidity	Temperature
PM _{2.5}	0.05	-0.05	-0.03
O ₃		-0.5	-0.06
Relative Humidity			0.1
Lag 2			
	O ₃	Relative Humidity	Temperature
PM _{2.5}	0.04	-0.01	-0.14
O ₃		-0.26	0.14
Relative Humidity			-0.1
Lag 3			
	O ₃	Relative Humidity	Temperature
PM _{2.5}	0.25	0.18	0.18
O ₃		-0.19	0.26
Relative Humidity			0.21
Lag 4			
	O ₃	Relative Humidity	Temperature
PM _{2.5}	0.42	-0.38	0.24
O ₃		-0.47	0.21
Relative Humidity			-0.05
Lag 5			
	O ₃	Relative Humidity	Temperature
PM _{2.5}	-0.31	-0.52	0
O ₃		-0.32	0.23
Relative Humidity			-0.34

G5. Detailed model results

Table G3. The model results of Figure 24.

	aMT6s			
	Effect size	CI (lower)	CI (upper)	P value
PM _{2.5}				
Lag 0	17.0	-1.9	39.5	0.081
Lag 1	16.8	-3.8	41.8	0.115
Lag 2	26.4	0.3	59.3	0.047
Lag 3	13.7	-5.7	37.0	0.176
Lag 4	-1.1	-17.4	18.5	0.905
Lag 5	13.4	-11.2	44.7	0.311
O ₃				
Lag 0	3.4	-16.0	27.3	0.751
Lag 1	23.0	-1.0	52.8	0.061
Lag 2	28.5	6.6	54.9	0.009
Lag 3	30.5	9.0	56.3	0.004
Lag 4	6.0	-8.5	22.9	0.432
Lag5	40.7	13.2	74.8	0.002

Table G4. The model results of Figure 25.

Biomarker	MDA				8-OHdG			
	Effect size	CI (lower)	CI (upper)	P value	Effect size	CI (lower)	CI (upper)	P value
aMT6s	73.4	52.6	97.0	<0.001	41.7	22.8	63.4	<0.001

Table G5. The model results of Figure 26.

	aMT6s			
	Effect size	CI (lower)	CI (upper)	P value
Z ₅	2.0	-2.1	6.1	0.341
R ₅	3.4	-1.5	8.4	0.175
R ₂₀	1.0	-3.5	5.5	0.654
R ₅ -R ₂₀	6.1	-5.8	17.9	0.314
X ₅	-11.6	9.6	-32.8	0.283
Fres	-8.1	-14.3	-1.8	0.012
FEV ₁	0.4	-1.5	2.4	0.676
FVC	0.3	-1.2	1.8	0.680
FEV ₁ /FVC	0.0	-1.3	1.4	0.981
FEF ₂₅₋₇₅	0.9	-3.5	5.4	0.704
PEF	1.0	-1.9	3.9	0.501
FeNO	-8.1	-15.5	-0.1	0.047

G6. Copollutant models

Copollutant models were developed following *Formula G2*.

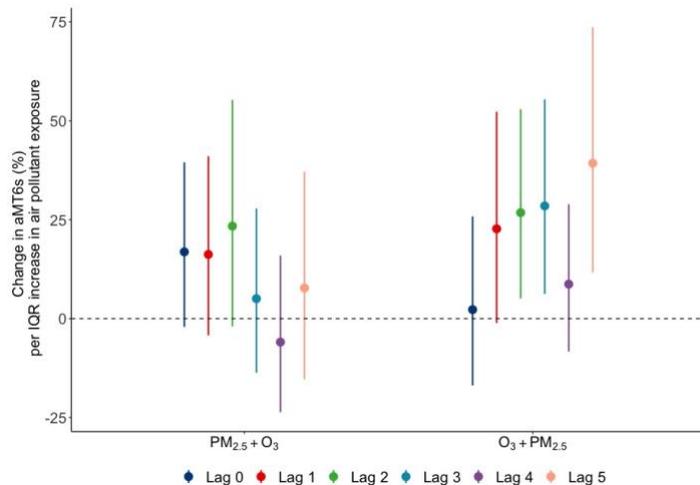


Figure G2. Estimated means and 95% confidence intervals for change in urinary aMT6s (%) one IQR increase in 24-hour average $PM_{2.5}$ exposure and maximum daily 8-hour average O_3 personal exposure 0 to 5 days prior to clinic visits (lag 0-5 days). Copollutant Models

G7. Sensitivity analyses

Sensitivity analyses were conducted for the associations shown in the main content in a dataset excluding measurements of participants who had asthma exacerbation during the two weeks prior to the clinical visits (7 measurements from 6 participants) following *Formula G1, G3, and G4*. The results are shown in Figure G3-G5.

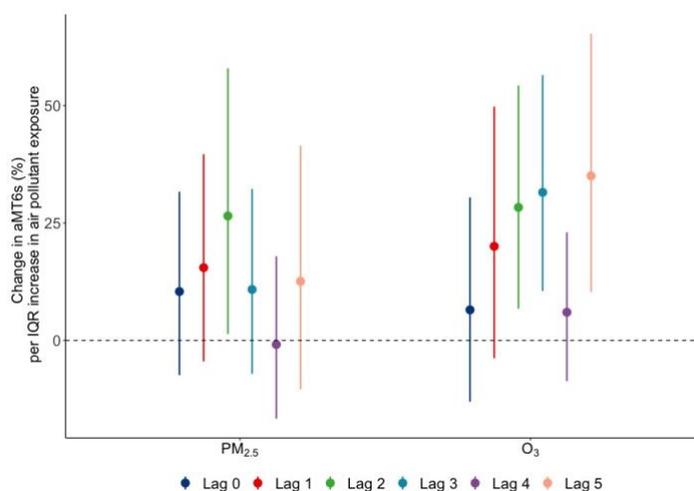


Figure G3. Estimated means and 95% confidence intervals for change in urinary aMT6s (%) one IQR increase in 24-hour average PM_{2.5} exposure and maximum daily 8-hour average O₃ personal exposure 0 to 5 days prior to clinic visits (lag 0-5 days).

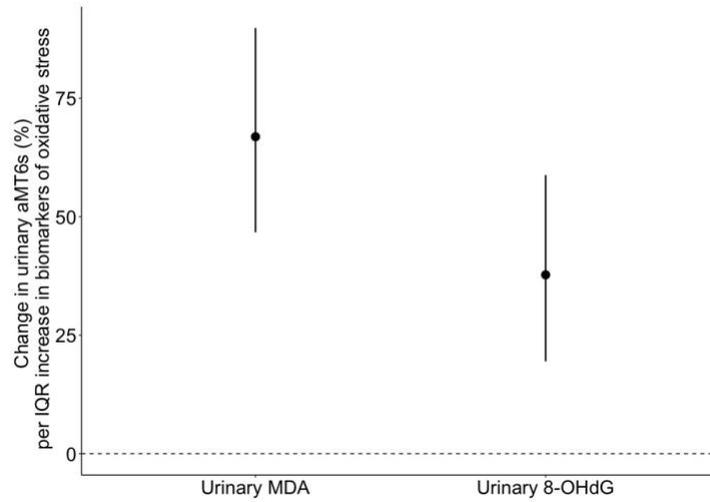


Figure G4. Estimated means and 95% confidence intervals for change in urinary aMT6s (%) one IQR increase in urinary MDA or 8-OHdG.

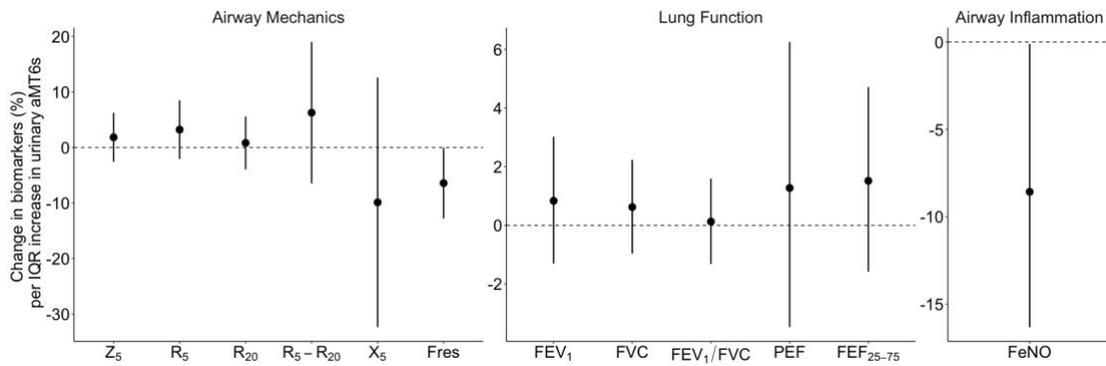


Figure G5. Estimated means and 95% confidence intervals for change in pulmonary health outcomes (%) one IQR increase in urinary aMT6s.

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Biography

Linchen He received Bachelor's degree in Environmental Engineering in 2013 from Shanxi University and Master's degrees in Environmental Management in 2015 and Environmental Sciences in 2020 from Duke University. Linchen Started his doctoral program at Duke University in 2016 under the supervision of Dr. Junfeng (Jim) Zhang. He was the recipient of James B. Duke Fellowship in 2016, Jacobs Graduate Research Scholarship in 2017, and Duke Global Health Doctoral Scholars Fellowship in 2019. During his graduate studies, Linchen has published seven first-author manuscript and is the co-author of other eight research articles.

Publications

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3. **He, L.;** Li, Z.; Teng, Y.; Cui, X.; Barkjohn, K. K.; Norris, C.; Fang, L.; Lin, L.; Wang, Q.; Zhou, X.; Hong, J.; Li, F.; Zhang, Y.; Schauer, J. J.; Black, M.; Bergin, M.; Zhang, J., Associations of personal exposure to air pollutants with airway mechanics in children with asthma. *Environment International* **2020**, 138, 105647.
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