

# Prenatal Exposure to Ambient Air Multi-Pollutant Mixture Significantly Impairs Intrauterine Fetal Development Trajectory

**Xiaowen Shao**

Shanghai Tenth People's Hospital

**Haoxiang Cheng**

Icahn School of Medicine at Mount Sinai <https://orcid.org/0000-0002-1393-6006>

**Jonathan Zhou**

Icahn School of Medicine at Mount Sinai

**Jushan zhang**

Shanghai Tenth People's Hospital

**Yujie Zhu**

Shanghai Tenth People's Hospital

**Chun Yang**

Shanghai Tenth People's Hospital

**Antonio Di Narzo**

Icahn School of Medicine at Mount Sinai

**Jing Yu**

Shanghai Tenth People's Hospital

**Yuan Shen**

Shanghai Tenth People's Hospital

**Yuanyuan Li**

Huazhong University of Science and Technology

**Shunqing Xu**

Huazhong University of Science and Technology

**Zhongyang Zhang**

Icahn School of Medicine at Mount Sinai

**Jia Chen**

Icahn School of Medicine at Mount Sinai

**Jiajing Cheng**

Shanghai Tenth People's Hospital

**Ke Hao** (✉ [ke.hao@mssm.edu](mailto:ke.hao@mssm.edu))

<https://orcid.org/0000-0002-1815-9197>

## Research

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# Abstract

Background Impaired *in utero* fetal growth trajectory may have long term health consequences of the newborns and increase risk of adulthood metabolic diseases. Prenatal exposure to air pollution has been linked to fetal development restriction; however, the impact of exposure to ambient air pollutants on the entire course of intrauterine fetal development has not been comprehensively investigated.

Methods During 2015 – 2018, two cohorts of mother-infant dyads (N=678 and 227) were recruited in Shanghai China, from which three categories of data were systematically collected: (1) daily exposure to six air pollutants during pregnancy, (2) fetal biometry in the 2<sup>nd</sup> (gestational week 24, [GW24]) and 3<sup>rd</sup> trimester (GW36), and (3) neonatal outcomes at birth. We investigated the impact of prenatal exposure to a mixture of air pollutants on the trajectory of fetal development during the course of gestation, adjusting for a broad set of potential confounds.

Results Prenatal exposure to PM<sub>2.5</sub>, PM<sub>10</sub>, SO<sub>2</sub> and O<sub>3</sub> significantly reduced fetal biometry at GW24, where SO<sub>2</sub> had the most potent effect. For every 10 µg/m<sup>3</sup> increment increase of daily SO<sub>2</sub> exposure during the 1<sup>st</sup> trimester shortened femur length by 2.20 mm (p=6.7E-21) translating to 5.3% reduction from population average. Prenatal air pollution exposure also decreased fetal biometry at GW36 with attenuated effect size. Comparing to the lowest exposed quartile, fetus in the highest exposed quartile had 6.3% (p=3.5E-5) and 2.1% (p=2.4E-3) lower estimated intrauterine weight in GW24 and GW36, respectively; however, no difference in birth weight was observed, indicating a rapid catch-up growth in the 3<sup>rd</sup> trimester.

Conclusions To our knowledge, for the first time, we demonstrated the impact of prenatal exposure to ambient air pollutants on the course of intrauterine fetal development. The altered growth trajectory and rapid catch-up growth in associated with high prenatal exposure may lead to long-term predisposition for adulthood metabolic disorders.

## Introduction

Impaired *in utero* fetal growth represents one of the greatest public health threats worldwide. The World Health Organization (WHO) estimated that > 20 million LBW babies are born each year (15% to 20% of all births), nearly 95% of them in developing countries

([https://www.who.int/nutrition/topics/globaltargets\\_lowbirthweight\\_policybrief.pdf](https://www.who.int/nutrition/topics/globaltargets_lowbirthweight_policybrief.pdf)). It is now accepted that restricted fetal growth and low birth weight (< 2500 g) may result in rapid catch-up growth which is associated with an increased risk of multiple diseases in adulthood, including hypertension, obesity, cardiovascular diseases, diabetes, and cancers (Zhenget al. 2016). This phenomenon is referred as the Developmental Origins of Health and Diseases (DOHaD) paradigm (Barker 1997; 2004; Barker and Godfrey 2001). Identification of the risk factors that alter fetal growth trajectory will provide prevention opportunities towards improving children's health and reducing risk of many adult diseases. While tremendous efforts have been made to understand these risk factors, results from epidemiologic studies

have been inconsistent and the environmental factors that may induce human suboptimal fetal growth and rapid catch-up growth are currently unclear.

In the recent decades, several birth cohort studies have evaluated prenatal exposure to ambient air pollutants including nitrogen dioxide (NO<sub>2</sub>), particulate matter (PM), and polycyclic aromatic hydrocarbons (PAHs) and fetal growth but yielded divergent results (Vieira 2015). Van den Hooven et al reported prenatal NO<sub>2</sub> and PM<sub>10</sub> exposure reduced birth weight, birth length and head circumference, but no significant effect on gestational age (van den Hooven et al. 2012). Gehring et al found no associations between prenatal PM exposure and BW in a Dutch prospective cohort (Gehring et al. 2011). In a multicenter study conducted in Spain, NO<sub>2</sub> exposure during pregnancy was found to reduce birth length, but benzene exposure had no effect on fetal development (Estarlich et al. 2011). A meta-analysis of 25 observational studies reported that an increase of 10ug/m<sup>3</sup> prenatal PM<sub>10</sub> exposure was associated with 14.8g decrease in birth weight (Zhu et al. 2015).

Beyond neonatal outcomes, intrauterine fetal biometry assessed by ultrasound also has been examined for association with air pollutants exposure, but most of the existing literatures focused on NO<sub>2</sub>. In a Spanish study, exposure to NO<sub>2</sub> was inversely associated with biparietal diameter (BPD) and estimated fetus weight between weeks 20 and 32 of gestation (Iniguez et al. 2012). In the same cohort, a follow-up analysis on traffic related air pollution (TRAP) reported NO<sub>2</sub> and aromatic hydrocarbon exposure was inversely associated with BPD growth during 20 to 32 weeks of gestation, but no relationships noted with other fetal growth parameters (Aguilera et al. 2010). The MAPSS (Maternal Air Pollution in Southern Sweden) cohort estimated negative effects for prenatal NO<sub>x</sub> exposure: decrease of abdominal diameter and femur length (FL) by -0.10 (-0.17, -0.03) and -0.13 (-0.17, -0.01) mm, respectively, per 10 µg/m<sup>3</sup> increment of NO<sub>x</sub> (Malmqvist et al. 2017).

China is one of the regions in the world of severe ambient air pollution (Wang et al. 2017; Xu et al. 2016). PM<sub>2.5</sub>, PM<sub>10</sub>, SO<sub>2</sub> and O<sub>3</sub> have been reported in association with risks of preterm birth in a cohort of Guangdong province, China, but was not statistically significant after multiple testing adjustment. In a Shanghai China cohort, after adjusting for other pollutants, 10 µg/m<sup>3</sup> increment in prenatal PM<sub>2.5</sub> exposure reduced fetal abdominal circumference (AC), BDP and FL by 5.48mm, 5.57mm and 5.47mm, respectively (Cao et al. 2019). However, the impact of prenatal PM<sub>2.5</sub> exposure on the fetus growth trajectory during the course of gestation has not been investigated.

To date, our understanding of prenatal exposure to ambient air pollutant mixture and fetus development at various gestational stages is incomplete. Most studies only investigate selected pollutants (e.g. NO<sub>2</sub> and PM<sub>2.5</sub>) and outcome at a single time point during gestation. In order to fill in such knowledge gaps, we established human cohorts in Shanghai, China and collected three categories of data (1) daily exposure to six air pollutants during pregnancy [3 months prior to conception – birth]; (2) fetal biometry assessed by ultrasound in the 2<sup>nd</sup> (gestational week 24 [GW24]) and 3<sup>rd</sup> trimester (GW36); and (3) neonatal outcomes at birth. We are positioned to characterize the impact of prenatal exposure to

multiple ambient air pollutant measurements on the trajectory of *in utero* fetal development in a comprehensive manner.

## Materials And Methods

### *Discovery cohort*

From the subjects who received obstetricians/gynecologists (OB/GYNs) care and delivered infants in the Shanghai Tenth People's Hospital during 2015-2018, we enrolled mother-infant dyads met the following inclusion/exclusion criteria into the discovery cohort. The subject resided in Shanghai, China and vicinity area three month before and throughout pregnancy. We excluded subjects with infectious disease, thyroid diseases, psychiatric disease, cognitive disorders, malignancies, severe kidney or liver dysfunction, severe or moderate preterm birth (<36 week of gestational age), and major birth defect. We also excluded smokers and infants of assisted reproductive technology and *in vitro* fertilization. We excluded mothers whose pre-pregnancy body mass index < 18. From the medical record database in the Shanghai Tenth People's Hospital, we retrieved de-identified data and preformed chart review, and obtained three batches of variables (1) mother's residential address, demographic and clinical parameters, (2) fetus biometry at GW24 and GW36 assessed by ultrasound, and (3) infant dimension measures at birth. The study was approved by the Institutional Review Boards at the Shanghai Tenth People's Hospital, Shanghai, China.

### *Replication cohort*

In order to validate the discovery cohort findings, we obtained de-identified data of additional 227 mother-infant dyads from Shanghai Tenth People's Hospital medical record database. The inclusion/exclusion criteria and chart review methods were identical as in the discovery cohort. The data access and analysis were approved by the Institutional Review Boards at Shanghai Tenth People's Hospital, Shanghai, China.

### *Assessment of fetus biometry*

Fetal biometry parameters: biparietal diameter, femur length, humerus length (HL), abdominal circumference were assessed at two time points, GW24 and GW36, by ultrasound during routine OB/GYN visit. BPD measures the diameter of the fetus skull, femur length measures the fetal leg and humerus length measures the fetal arm length. All measurements were conducted three times, and their average were used in data analysis. After data retrieval and chart review we conducted careful data QC to verify/correct suspicious values by manually confirming the medical chart.

### *Estimating fetal weight*

We estimate the weight of fetus, as well as the head and trunk weight, using formula published by Shinozuka et al (Shinozuka et al. 1987): fetus weight =  $\text{weight}_{\text{head}} + \text{weight}_{\text{trunk}} = 1.07 \times \text{BPD}^3 + 0.3 \times \text{AC}^2 \times \text{FL}$ , where weight was in gram, and BPD, AC and FL were in cm.

## ***Estimating ambient air pollutant exposure level***

From two sources, we obtained air pollutant concentration at each subject's residence address.

(1) Ambient air pollutant data (hourly concentrations) is published by 22 MEE (Ministry of Ecology and Environment of China) monitoring stations in Shanghai, China and vicinity area, from 2014 to 2018 (<http://beijingair.sinaapp.com/>). The latitude and longitude of each station was also known. In parallel, the residential addresses of the participants were geocoded into latitude and longitude using google map, and pollutant concentration of the closest station to each individual was assigned as her exposure level. In total, 11 MEE stations were the closest station to at least one participant (Table S1), and data from these stations were used in this study.

(2) Real-time and high geographical granularity air quality data from UrbanAir (Zhenget al. 2013). UrbanAir compute pollutant levels based on the historical and real-time air quality data reported by monitor stations, meteorology information, traffic flow, human mobility, and structure of road networks. We map each study subject's address to a 1km ´ 1km grid, and assign the UrbanAir reported pollutant concentration of the particular grid as her exposure level. We found the pollutant data from source (1) and (2) are highly consistent.

In total, six pollutants were investigated: SO<sub>2</sub>, PM<sub>2.5</sub>, PM<sub>10</sub>, O<sub>3</sub>, NO<sub>2</sub>, and CO. We computed each participant's average exposure level of four gestational periods, pre-conception (**PC**): three months prior to conception, first trimester (**T1**): 0-13 week in pregnancy, second trimester (**T2**): 14-26 week in pregnancy, third trimester (**T3**): 27 week – delivery.

## ***Data Analysis***

All data analysis was conducted using R statistical software (version 3.6.2). Pearson correlation were computed among pollutants and among fetus/newborn biometry. Hierarchical clustering was performed using *hclust* function in R.

First, we tested the impact of prenatal air pollution exposure to fetus development in the discovery cohort, second, we validated results in replication cohort using identical models. In detail, we fit robust linear model (rlm):  $Y = E + Z$  (Model 1), where Y denote the fetus/infant biometry of interest, E denote the exposure of a given pollutant, and Z denote covariables including mother's age, height, occupation, birth calendar quarter, gravidity, parity, method of delivery, and exposure to other pollutants. The model was fit for each outcome – pollutant – gestational period combination separately. Given the six pollutants formed two clusters (Fig S1) and the Cluster 1 pollutants (SO<sub>2</sub>, PM<sub>2.5</sub>, PM<sub>10</sub>, NO<sub>2</sub>, and CO) were highly correlated (ie, co-linearity), we constructed principle components for Cluster 1 in each of the gestational period (PC, T1, T2 and T3), where first principle component explained 79%-83% variability. In testing the effect of Cluster 1 pollutant, we adjusted O<sub>3</sub> in the model, and in testing the effect of O<sub>3</sub>, we adjusted first principle component of Cluster 1.

# Results

## *Ambient air pollutant level*

From two sources, we obtained hourly levels of 6 pollutants from 2015 – 2018 (Materials and Methods), and the average pollutant concentration during study period were summarized in Table S1. The PM<sub>2.5</sub> monthly average ranged between 29.48 and 64.79 ug/m<sup>3</sup>; and PM<sub>10</sub> monthly average ranged between 46.32 and 88.63 ug/m<sup>3</sup> (Table S1). The pollutant levels showed both spatial and temporal patterns. In general, the air quality was better in the east part than the west part of the city. Focused on the monitoring stations which is closest to at least one patient (11 stations in total, Table S1), the average PM<sub>10</sub> and PM<sub>2.5</sub> levels of the stations east of Huangpu river were 8.4% and 6.3% lower, respectively, than the stations west of Huangpu river during study period (Table S1). Meanwhile, the daily average pollutant levels of the 11 MEE stations were highly correlated (Fig S2). Such spatial correlation was more substantial in particulate matters than in gas pollutants. For example, the mean correlation of PM<sub>2.5</sub> among stations were 0.95, and mean correlation of CO among stations were 0.72.

Clear temporal pattern is also observed, all pollutants except O<sub>3</sub> had higher concentration in the 1<sup>st</sup> and 4<sup>th</sup> calendar quarters than the other two quarters, in contrast, O<sub>3</sub> showed reverse pattern (Table S1 and Fig 1). The concentration of PM<sub>2.5</sub>, PM<sub>10</sub>, CO and SO<sub>2</sub> trended downward consistently from 2015 to 2018 (Fig 1), primarily attributable to increasingly stringent clean air policy in China (Zhanget al. 2019). However, the concentration of NO<sub>2</sub> and O<sub>3</sub> were largely unchanged during 2015-2018. Hierarchical clustering grouped the six pollutants into two clusters (Fig 2). Cluster 1 included SO<sub>2</sub>, PM<sub>2.5</sub>, PM<sub>10</sub>, NO<sub>2</sub>, and CO where the mean inter-pollutant correlation was 0.72. Cluster 2 contained O<sub>3</sub>, which had negative correlation with cluster 1 pollutants.

## *Demographic and clinical characteristics*

A total of 678 and 227 mother-infant dyads were recruited to the discovery and replications cohorts, respectively. The two cohorts are comparable in terms of fetal/neonatal biometry (Table 1) and maternal characteristics (Table S2). The replication had more female (55.1%) than in discovery cohort (45.6%), and had slighted smaller head circumference: 33.6cm vs. 33.8 cm, p=0.005 (Table 1). The mothers' average age at delivery was 29.0 and 29.2 years in the discovery and replication cohorts. 64.9% and 63.9% of the delivery in discovery and replication cohorts were vaginal (Table S2). Based on gestational age (GA) and observed newborn dimensions: birth length (BL), birth weight (BW) and head circumference (HC), we computed fetus growth rate: BL/GA, BW/GA and HC/GA (Table 1).

We pooled the cohorts in hierarchical clustering analysis, which revealed the correlation architecture among the fetus and infant biometry (Fig 2). The traits measured at each gestational stage (GW24, GW36 and birth) grouped into clusters. For example, GW24 biometry (BDP, FL, HL and AC) formed a cluster, and so did the GW36 biometry. Importantly, gestational age is negatively correlated with GW24 and GW36 biometry, indicating prolonged gestation could be a mechanism to compensate for slow fetal

growth. We restricted hierarchical clustering analysis within the 585 mother-infant dyads of vaginal delivery and the findings remained unchanged (Fig S3).

### ***Fetal development was significantly influenced by prenatal ambient air pollutant exposure***

First, we examined the association between fetal/neonatal outcomes and prenatal exposure in the discovery cohort using Model 1 (Materials and Methods). The statistical tests were conducted on each outcome – pollutant - gestational period combination separately, resulted in a total of 336 tests, and false discover rate (FDR) was calibrated using Benjamini and Hochberg procedure (Benjamini and Hochberg 1995) to control multiple testing (Fig 3A and Table S4). Exposure to PM<sub>2.5</sub>, PM<sub>10</sub>, and SO<sub>2</sub> during PC, T1 and T2 periods significantly reduced GW24 FL, HL and AC (Fig 3A). For example, a 10 ug/m<sup>3</sup> increment of PM<sub>2.5</sub> exposure during the first trimester reduced FL by 0.839 mm (p=5.1E-12 and FDR=3.5E-10); a 10 ug/m<sup>3</sup> increment of PM<sub>10</sub> exposure during the first trimester reduced FL by 0.605 mm (p=1.0E-15 and FDR=2.1E-13) (Table S4). SO<sub>2</sub> showed the most profound effect size: a 10 ug/m<sup>3</sup> increase during T1 reduced FL by 2.11 mm (p=2.3E-15 and FDR=2.5E-13), translating to 5% reduction from the population average. In addition, pre-conception exposure to O<sub>3</sub> shortened GW24 FL and HL (FDR= 2.6E-3 and 4.7E-2, respectively). Exposure to air pollutant during all four gestational periods reduced GW36 FL and HL. Even a 10 ug/m<sup>3</sup> increment of PM<sub>2.5</sub>, PM<sub>10</sub>, SO<sub>2</sub> and O<sub>3</sub> exposure during pre-conception period reduced FL by 0.474 mm (FDR=1.9E-4), 0.246 mm (FDR=2.6E-4), 0.898 mm (FDR=2.8E-4), and 0.240mm (FDR= 2.9E-2) respectively, suggesting pollutant exposure may have long lasting effect during pregnancy. PM<sub>2.5</sub>, PM<sub>10</sub>, and SO<sub>2</sub> exposure were positively associated with gestational age, for example a 10 ug/m<sup>3</sup> increment of PM<sub>2.5</sub> exposure during the third trimester increased gestation by 0.924 days (FDR=0.02), possibly a compensation mechanism to fetal growth restriction caused by air pollution. Importantly, prenatal ambient air pollutant exposure was not associated with birth weight (Fig 3A).

Second, we successfully validated the initial findings in the replication cohort (Fig 3B and Table S5). Prenatal exposure to PM<sub>2.5</sub>, PM<sub>10</sub>, SO<sub>2</sub> and O<sub>3</sub> reduced FL, HL and AC at GW24, reduced FL and HL at GW36, and elongated gestation (Fig 3B and Table S5).

Third, we pooled the two cohorts and conducted joint analysis (Fig 3C and Table S6). Prenatal exposure to PM<sub>2.5</sub>, PM<sub>10</sub>, SO<sub>2</sub> and O<sub>3</sub> significantly reduced GW FL, HL and AC, where SO<sub>2</sub> had the most potent effect. A 10 ug/m<sup>3</sup> increment of daily SO<sub>2</sub> exposure during T1 shortened FL by 2.20 mm (FDR=6.4E-19) translating to 5.3% reduction from population average. A 10 ug/m<sup>3</sup> increment of PM<sub>2.5</sub> daily exposure during T1 shortened FL by 0.85 mm (FDR=6.7E-14). Importantly, air pollution did not affect BPD (fetal skull development), pointing to asymmetric growth restriction (fetus concentrates nutrition to head growth at the expense of trunk development) (Delpishehet al. 2008; Sharma et al. 2016a; Sharma et al. 2016b). At GW, FL and HL were significantly associated with prenatal PM<sub>2.5</sub>, PM<sub>10</sub>, SO<sub>2</sub> and O<sub>3</sub> exposure. A 10 ug/m<sup>3</sup> increment of daily exposure to SO<sub>2</sub> and PM<sub>2.5</sub> during T1 shortened FL by 1.07 mm (FDR=8.4E-5) and 0.536mm (FDR=7.2E-6), respectively (Table S6). Prenatal exposure to air pollution had

no effect on birth weight, but significantly associated with longer gestation, and lower body length growth rate (BL/GA) (Fig 2C).

### ***Impact of prenatal ambient air pollutant on fetal growth trajectory***

We rank the infants by their prenatal ambient air pollutants exposure levels. Since each subject was exposed to multiple types of pollutants (i.e, mixture exposure), we computed the relative potency, a strategy commonly used in the mixture exposure modeling (Luet al. 2018). The relative potency factor was assigned as the association coefficient between each pollutant and estimated GW fetal weight. Using the lowest exposed quartile as the reference group, fetus in the highest exposed quartile had 6.3% smaller estimated fetal weight ( $p=3.5E-5$ ) and 7.9% smaller fetal trunk weight ( $p=7.3E-9$ ) at GW24. Interestingly, estimated fetal head weight showed no difference between the lowest and the highest exposed quartiles (Fig 4 and Table S7), indicating an asymmetrical growth restriction and possible placental insufficiency mechanism (Delpishehet al. 2008; Sharmaet al. 2016a; Sharmaet al. 2016b). The estimated fetal weights of the two quartiles were still significantly different at GW36 with somewhat attenuated magnitude, where the highest exposed quartile had 2.1% ( $p=2.4E-3$ ) and 2.7% ( $p=3.3E-4$ ) lower body weight and trunk weight, respectively. The highest exposed quartile had 1.4 day longer gestation ( $p=0.0186$ ), but no significant difference in birth weight comparing to lowest exposed quartile, indicating a strong catch-up growth in the third trimester. To avoid the potential confounding of delivery methods, we repeated analysis only in infants of vaginal delivery and the findings remained unchanged (Table S8).

## **Discussion**

In the past two decades, multiple epidemiology studies have linked prenatal air pollution exposure and restricted fetus development. However, most of these studies have been conducted in Europe and North American, where the pollutant levels are much lower than in China. Further, the existing studies only examined selected pollutants (e.g.  $NO_2$ ) or at a single time point (e.g. at birth). To our knowledge, we are the first to simultaneously study multiple ambient air pollutants and full course of fetal developmental biometric parameters. Key findings of this study:

**(1)** Prenatal exposure to  $PM_{2.5}$ ,  $PM_{10}$ ,  $SO_2$  and  $O_3$  significantly reduced GW24 FL, HL and AC, where  $SO_2$  had the most potent effect. Also at GW36, FL and HL were significantly shortened by prenatal  $PM_{2.5}$ ,  $PM_{10}$ ,  $SO_2$  and  $O_3$  exposure. Prenatal exposure to air pollution had no effect on birth weight, but significantly associated with longer gestation, and slower body length growth rate (BL/GA) (Fig 3C).

**(2)** Comparing to the lowest exposed quartile, fetus in the highest exposed quartile had 6.3% ( $p=3.5E-5$ ) and 2.1% ( $p=2.4E-3$ ) lower estimated weight in GW24 and GW36, respectively, but no difference in birth weight, indicating a rapid catch-up growth in the 3<sup>rd</sup> trimester (Fig 4).

**(3)** The air pollution induced fetal growth restriction is strongly asymmetrical, where fetal trunk was affected more profoundly than fetal head (Fig 3 and Fig 4). The asymmetrical growth restriction is

caused by fetus preserves nutrition to head growth at the expense of trunk development in the scenario of placental insufficiency (Delpishehet al. 2008; Sharmaet al. 2016a; Sharmaet al. 2016b).

Our study characterized the impact of prenatal air pollutant exposure on the trajectory of *in utero* development: growth restriction during early-middle stages of pregnancy, followed by rapid catch-up during late pregnancy. Such altered trajectory may lead to serious health consequences. Vast literature linked catch-up growth with increased risk of adulthood, including hypertension, obesity, cardiovascular diseases, diabetes, and cancers (Adairet al. 2013; Mangel and Munch 2005; Singhal 2017; Singhal and Lucas 2004; Zhenget al. 2016). Such association has been seen in infants in both low- and high-income countries, in infants born preterm or at term, and those born with normal or low birth weight for gestation (Singhal 2017). Randomized trials of breast- and formula-fed infants support a causal link between early growth acceleration and infant nutrition and later risk of obesity. Our findings are of great public health importance that strategies to optimize the fetus growth trajectory could make a major contribution to prevent the current global epidemic of obesity and metabolic diseases (Singhal 2017).

In China and most parts of the world, a mix of ambient air pollutants that come from different sources or are created from different chemical reactions in the atmosphere. Critical science is being conducted to manage air quality in a more integrated manner to take into account emissions and exposures of multiple pollutants in the atmosphere, rather than single pollutants (Olstrupet al. 2019; Tajudinet al. 2019; Tanzeret al. 2019; Vedal and Kaufman 2011). We examined 6 pollutants which clearly formed two clusters (Fig S1). The Cluster 1 pollutants (SO<sub>2</sub>, PM<sub>2.5</sub>, PM<sub>10</sub>, NO<sub>2</sub>, and CO) were highly correlated. The first principle component Cluster 1 explained 79%-83% variability in the 4 pregnancy periods. From the mathematical viewpoint, such high co-linearity among variables make it very challenging to identify the pollutant-specific effect even with advanced mixture modeling methods (Bobbet al. 2015; Czarnotaet al. 2015). In this paper, we presented the effect of each pollutant separately (Fig 3), and future studies, e.g. animal model under controlled exposure, are necessary to dissect the pollutant specific and synergistic effects.

This study considered and controlled for an extensive set of potential confounders, including mother's age, height, occupation, birth calendar quarter (*i.e.* seasonal effect), gravidity, parity, method of delivery, and exposure to other pollutants. Cigarette smoking is known to restrict fetal growth (Pringleet al. 2005; Shisleret al. 2017), and we excluded smokers from cohort enrollment. In data QC and cleaning study, suspicious values were checked/re-confirmed by manual chart review. The statistical analysis employed robust linear model (rlm) which is resistant to extreme data point and ensure valid results. Over 35% of participants were caesarean section (Table S3), which could bias the gestational age and neonatal outcomes (e.g. birth weight) estimations. We repeated all analyses only among mother-infant dyads of vaginal delivery, and results were unchanged.

In an epidemiological study, we are not in a position to directly determine the biological mechanisms on how air pollution impairs fetal growth. Nevertheless, our observations provide plausible leads towards the etiological mechanisms. Exposure to air pollution, especially fine particles, has been shown to induce

oxidative stress and inflammation (Malmqvist et al. 2017; Terzano et al. 2010). Pregnant women have an approximately 50% increased alveolar ventilation rate (Hackley et al. 2007) resulting in an increased uptake of inhaled pollutants including ultrafine particles. When inhaled, fine particles can penetrate the alveolar wall and enter the maternal bloodstream such that particles and inflammatory mediators may reach the placenta and the fetus (Thompson 2018; Valavanidis et al. 2008). Further, placental mitochondrial DNA content has been suggested as a mediator between air pollution and birth weight (Clemente et al. 2016). It is well documented that cigarettes smoking causes placental insufficiency and in turn fetal growth restriction, and such restriction is often asymmetrical. In this study, we observed strong asymmetrical growth restriction at GW24 and GW36, where fetal trunk was affected more profoundly than fetal head. It is likely the fetus preserved nutrition to head growth at the expense of trunk development, pointing etiological mechanism that prenatal air pollution exposure impaired placenta function and constrained fetus access to nutrition (Delpisheh et al. 2008; Sharma et al. 2016a; Sharma et al. 2016b).

In summary, for the first time, we systematically studied the impact of prenatal exposure to six ambient air pollutants on fetal growth during the course of gestation. The study used two cohorts and convincingly showed that prenatal exposure to PM<sub>2.5</sub>, PM<sub>10</sub>, SO<sub>2</sub> and O<sub>3</sub> reduced GW24 and GW36 fetal biometry, but was not associated with birth weight, suggesting rapid catch-up at the late stage of pregnancy, which may predispose the infants to adulthood metabolic diseases. Moreover, the fetal growth restriction is also asymmetric, suggesting plausible etiological mechanism through placental insufficiency. From a public health perspective, our results provided much need evidence of prenatal air pollutant mixture exposure and impaired fetal development trajectory, and guidance in preventing both fetal growth restriction and long term health consequences.

## Abbreviations

<b>BPD</b>	biparietal diameter
<b>FL</b>	femur length
<b>HL</b>	humerus length
<b>AC</b>	abdominal circumference
<b>PC</b>	prior to conception
<b>T1</b>	first trimester
<b>T2</b>	second trimester
<b>T3</b>	third trimester
<b>GA</b>	gestational age
<b>BL</b>	birth length
<b>BW</b>	birth weight
<b>HC</b>	head circumference
<b>GW</b>	Gestational week
<b>PM</b>	Particulate matter

## Declarations

### Ethics approval and consent to participate

The Human Ethics Committee of Shanghai Tenth People's Hospital approved the study, and only de-identified data was used in the study.

### Consent for publication

All authors read and approved the final manuscript.

### Availability of data and materials

The datasets used in this study are available from the corresponding author upon reasonable request.

### Competing interests

No competing interests declared

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## **Authors' contributions**

Conceptualization: XS, CJ and KH;

Methodology: XS, HC, J.Zhou, J.Zhang, YZ, CY, ADN, ZZ and KH;

Formal Analysis: HC, J.Zhou, and KH

Resources and data collection: XS, HC, J.Zhou, J.Zhang, YZ, CY and CJ

Writing – Original Draft Preparation: KH;

Writing – Review and Editing: XS, HC, JY, YS, YL, SX and JC

Supervision: KH;

XS and HC contributed equally and are considered co-first authors.

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Not applicable

## **Author's Information**

Xiaowen Shao<sup>1\*</sup>, Haoxiang Cheng<sup>2\*</sup>, Jonathan Zhou<sup>2</sup>, Jushan Zhang<sup>3</sup>, Yujie Zhu<sup>3</sup>, Chun Yang<sup>3</sup>, Antonio Di Narzo<sup>2</sup>, Jing Yu<sup>4</sup>, Yuan Shen<sup>5</sup>, Yuanyuan Li<sup>6</sup>, Shunqing Xu<sup>6</sup>, Zhongyang Zhang<sup>2</sup>, Jia Chen<sup>7</sup>, Cheng Jiajing<sup>1†</sup>, Ke Hao<sup>2,3,8†</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, Shanghai Tenth People's Hospital, Tongji University, Shanghai, China

<sup>2</sup>Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA

<sup>3</sup>Department of Respiratory Medicine, Shanghai Tenth People's Hospital, Tongji University, Shanghai, China

<sup>4</sup>Department of Ophthalmology, Shanghai Tenth People's Hospital, Tongji University, Shanghai, China

<sup>5</sup>Department of Psychiatry, Shanghai Tenth People's Hospital, Tongji University, Shanghai, China

<sup>6</sup>Key Laboratory of Environment and Health, Ministry of Education & Ministry of Ecology and Environment, and State Key Laboratory of Environmental Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China

<sup>7</sup>Department of Environmental Medicine and Public Health, Icahn School of Medicine at Mount Sinai, New York, NY, USA

<sup>8</sup>[College of Environmental Science and Engineering](#), Tongji University, Shanghai, China

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# Supplemental Figures

**Legend to Fig S1:** Pearson correlation (r) and hierarchical clustering among fetus biometry and birth outcomes in 585 mother-infant dyads of vaginal delivery.

**Legend to Fig S2.** Pearson's correlation of ambient air pollutant (daily average value) in 11 MEE stations in Shanghai. Mean correlation in SO<sub>2</sub>, PM<sub>2.5</sub>, PM<sub>10</sub>, O<sub>3</sub>, NO<sub>2</sub>, and CO, are 0.84, 0.95, 0.93, 0.86, 0.84 and 0.72, respectively.

**Legend to Fig S3:** Pearson correlation (r) and among daily concentration among six major ambient air pollutants.

## Table

Table 1. Fetal biometry and infant birth outcomes

Variable	Discovery Cohort (n = 678)	Replication Cohort (n = 227)	p-value
Infant gender (female, %)	45.6	55.1	0.016
<b>Week 24 fetal biometry, mm</b>			
Biparietal diameter	61.6±2.97	61.2±2.97	0.050
Femur length	41.7±2.97	41.1±2.97	0.214
Humerus length	38.7±2.97	38.4±2.97	0.173
Abdominal circumference	199.9±13.34	199.8±14.83	0.098
<b>Week 36 fetal biometry, mm</b>			
Biparietal diameter	90.8±2.97	90.7±2.97	0.383
Femur length	67.5±2.97	67.2±2.97	0.437
Humerus length	58.8±1.48	58.6±2.97	0.121
Abdominal circumference	317.1±8.9	317.3±11.86	0.825
Gestational age (GA), day	279.7±5.93	280.3±7.41	0.345
Birth length (BL), cm	50.1±0.0	50.1±0.0	0.931
Birth weight (BW), gram	3422±355.82	3405.7±355.82	0.578
Head circumference (HC), cm	33.8±1.48	33.6±1.48	0.005
<b>Fetus growth rate</b>			
BL/GA, cm/day	0.2±0.0	0.2±0.0	0.301
BW/GA, gram/day	12.2±1.2	12.2±1.17	0.411
HC/GA, cm/day	0.1±0.0	0.1±0.0	0.004

\* In quantitative traits, mean ± median absolute deviation (MAD) are presented. Wilcoxon rank test and Chi square tests were applied to examine the differences for quantitative and categorical traits, respectively.

## Figures

Fig 1.

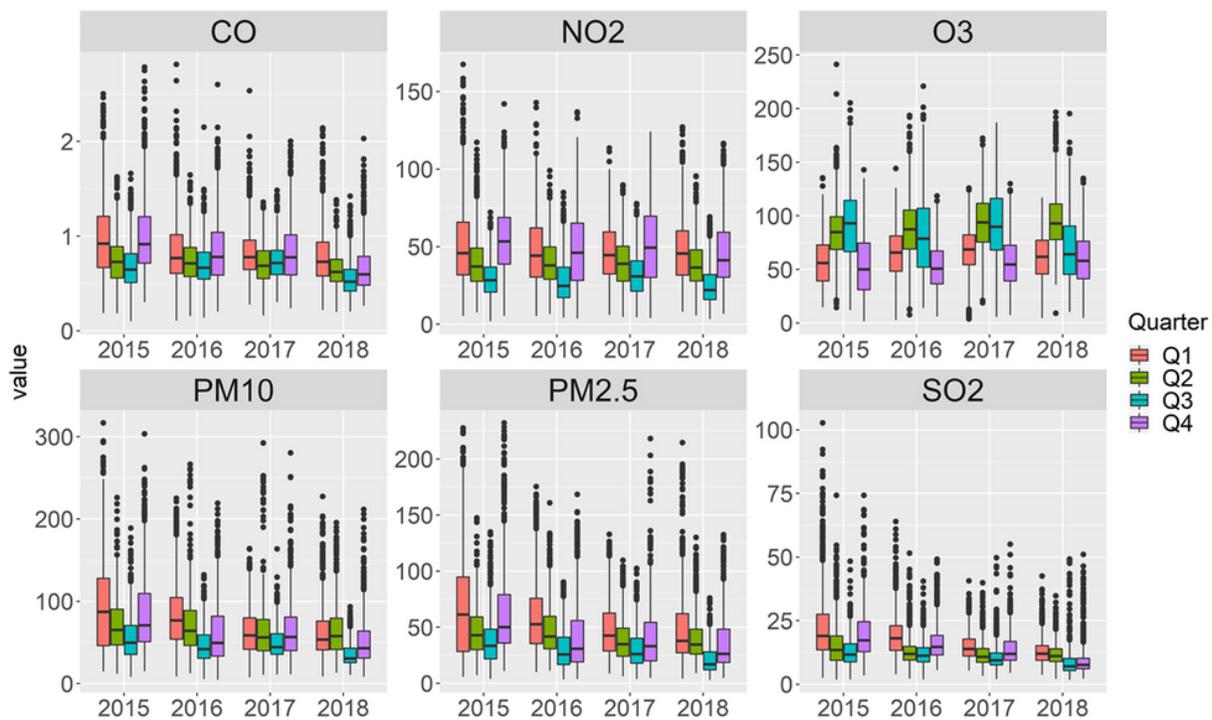
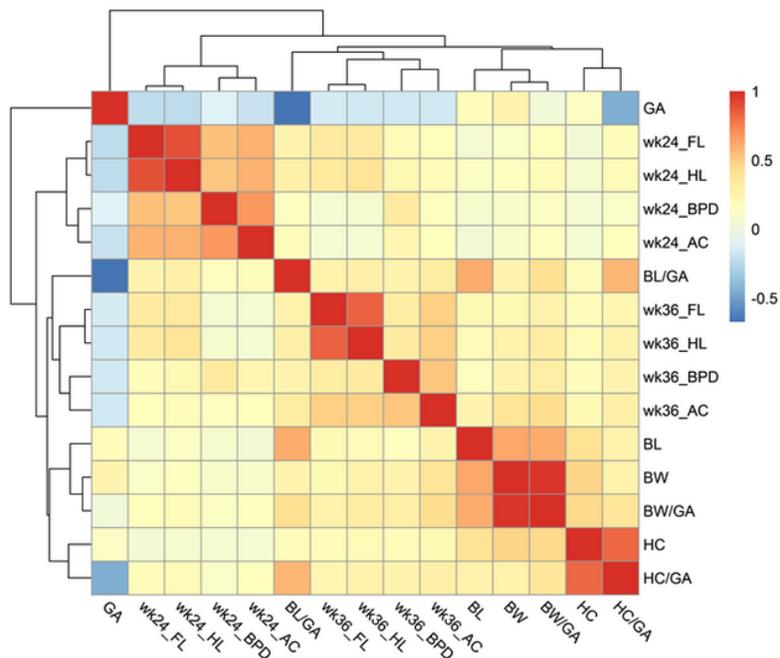


Figure 2

Distribution of daily average of six major ambient air pollutants in Shanghai during study period by calendar quarter.

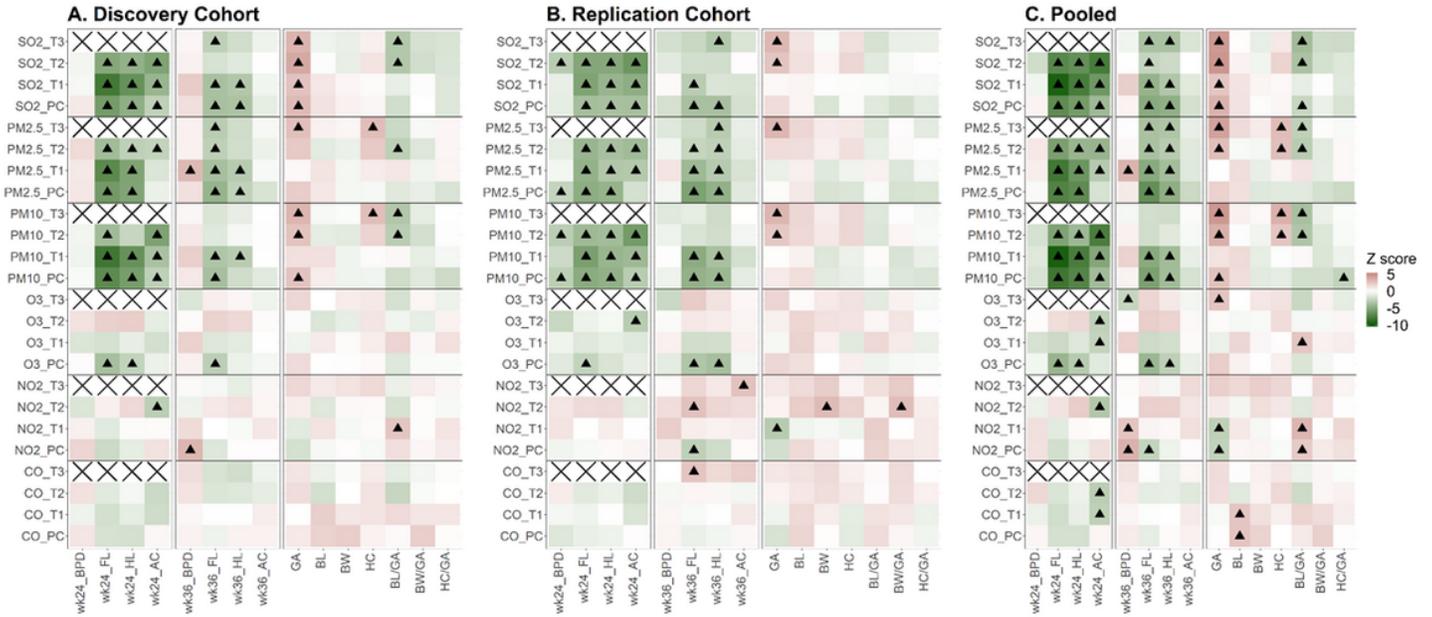
Fig 2.



**Figure 4**

Pearson correlation ( $\rho$ ) and hierarchical clustering among fetus biometry and birth outcomes.

Fig 3.



**Figure 6**

Association Z score between ambient air pollutants during pregnancy and fetus development in discovery cohort and replication cohort. PC, three months before estimated conception time point; T1, first trimester: 0-13 week in pregnancy; T2, second trimester: 14-26 week in pregnancy; T3, third trimester: 27 week – delivery. The association test was adjusted for covariables including mother’s age, mother’s height, mother’s occupation, birth quarter (calendar quarter), gravidity, parity, method of delivery, and exposure to other pollutants. Significant level was calibrated using Benjamini Hochberg FDR. A, Z score in discovery cohort; B, Z score in replication cohort; C, Z score on pooled sample (discovery and replication cohorts). In each panel, black triangle indicates  $FDR \leq 5\%$ ;

Fig 4.

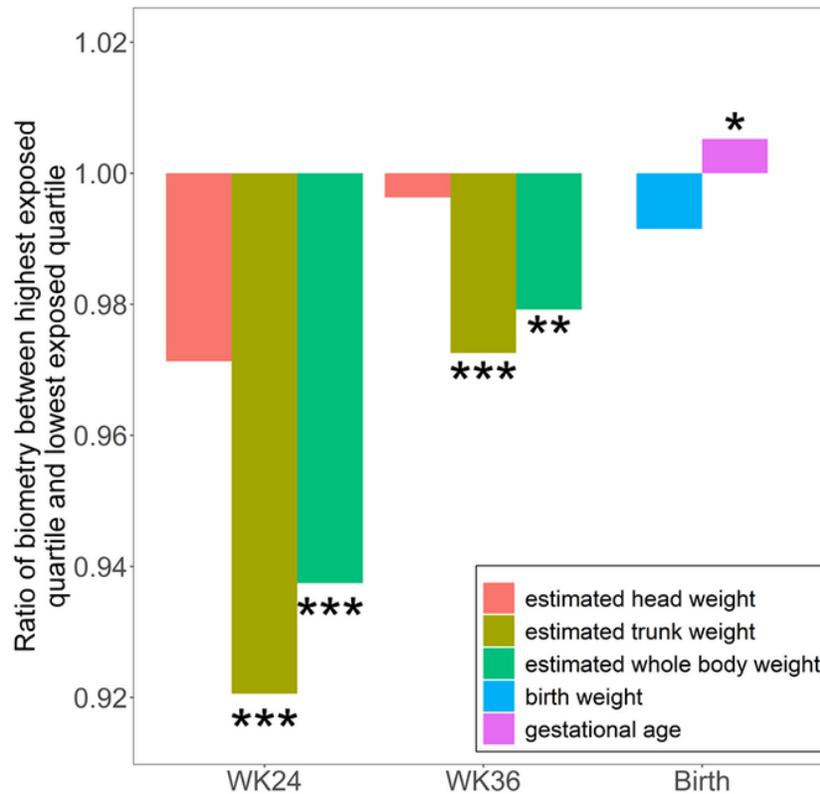


Figure 8

Infants were ranked by prenatal exposure level, and we compared the highest exposed quartile and lowest exposed quartile (ie, reference group). Fetus weight was estimated using formula described in Materials and Methods. \*:  $p$ value  $\leq 0.05$ ; \*\*:  $p$ value  $\leq 0.01$ ; \*\*\*:  $p$ value  $\leq 0.001$ .

## Supplementary Files

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