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A prospective study (SCOPE) comparing the cardiometabolic and respiratory effects of air pollution exposure on healthy and pre-diabetic individuals

Yanwen Wang[1†], Yiqun Han[1†], Tong Zhu[1*], Weiju Li[2] & Hongyin Zhang[2]

[1]*BIC-ESAT and SKL-ESPC, College of Environmental Sciences and Engineering, Peking University, Beijing 100871, China;*
[2]*Peking University Hospital, Peking University, Beijing 100871, China*

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Air pollution is known to be a major risk factor for cardiopulmonary disease, but this is unclear for cardiometabolic disease (e.g. diabetes). This is of considerable public health importance, given the nationwide epidemic of diabetes, accompanied by severe air pollution, in China. The evidence so far remained inadequate to answer questions of whether individuals with cardiometabolic dysfunctions are susceptible to air pollution and whether air pollution exacerbates diabetes development via certain biological pathways. In this manuscript, we summarize the results and limitations of studies exploring these two topics and elaborate our design of a prospective panel study (SCOPE) as a solution. We assessed and compared the health effect of air pollution among pre-diabetic individuals and matched healthy controls through four repeated clinical visits over 1 year. Comprehensive evaluation was made to both health endpoints and exposure. The primary biomarkers were assessed to reveal the impact on multiple biological pathways, including glycolipid metabolism and insulin resistance, endothelial function, and inflammation. Detailed chemical and size fractional components of particulate matter were measured in this study, along with the application of personal monitors. The work should increase our understanding of how air pollution affects individuals with cardiometabolic dysfunction and the underlying mechanisms.

panel study, air pollution, diabetes, susceptibility, cardiometabolic effect

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INTRODUCTION

The Global Burden of Diseases (GBD) study reported that exposure to ambient particulate matter was the fourth-leading risk factor for premature mortality in China (Yang et al., 2013). We earlier combined data from the national monitoring network with those of regional air quality models to estimate that, nationally, premature mortality attributable to ambient fine particulate matter of aerodynamic diameter ≤2.5 μm ($PM_{2.5}$) was 1.37 million annually (Liu et al., 2016). This estimation, based on the integrated evidence from toxicology and epidemiology studies, was composed primarily of cardiopulmonary disease. However, this figure may be an underestimate, as we did not consider the impact of air pollution on diseases associated with cardiometabolic dysfunction, such as type 2 diabetes (T2D).

In 2010, the age-standardized prevalence of total diabetes and pre-diabetes in China were 11.6% and 50.1% (113.9 and 493.4 million adults), respectively (Xu et al., 2013). A recent,

†Contributed equally to this work
*Corresponding author (email: tzhu@pku.edu.cn)

prospective nationwide study found that diabetic adults were at significantly increased risk of mortality from a variety of cardiovascular and non-cardiovascular diseases (Bragg et al., 2017). As the numbers of diabetics are large and the disease burden significant, the effects of any small risk factor (such as air pollution) will be magnified. Therefore, two questions arise: Is an individual with cardiometabolic dysfunction more susceptible to air pollution? Does air pollution exacerbate the progression of cardiometabolic disease?

Recent studies implied that air pollution contributes to T2D development. A cross-sectional study performed in the USA found that the prevalence of T2D increased by 1% with every 10 μg m^{-3} increase in $PM_{2.5}$ exposure (Pearson et al., 2010). Cohort studies performed in Germany (Krämer et al., 2010) and the USA (Coogan et al., 2012) suggested that long-term exposure to traffic-related pollutants ($PM_{2.5}$ and NO_2) increased the risk of T2D in females. Cohort studies performed in Canadian adults reported that even low levels of $PM_{2.5}$ exposure increased the risks of both T2D (Chen et al., 2013) and mortality (Brook et al., 2013).

A few studies also explored the underlying mechanisms. Positive associations were observed between daily exposure to BC and $PM_{2.5}$ and the levels of markers of endothelial dysfunction in patients with T2D (O'Neill et al., 2007). Cross-sectional studies performed in Taiwan found that the level of 1-year exposure to PM_{10} was associated with the extents of glucose and lipid metabolic disorders, including elevations in total cholesterol, triglycerides, fasting glucose, and HbA1c, in both younger adults and elderly people (Chuang et al., 2010; Chuang et al., 2011). In children in Iran, the 7-day extent of exposure to PM_{10} was significantly associated with rises in the levels of biomarkers of inflammation, oxidative stress, and insulin resistance, even after adjustment for multiple potential confounding factors (Kelishadi et al., 2009). A few prospective studies have suggested that both fine and coarse PMs exert adverse effects on HOMA-IR, insulin, triglycerides, and LDL levels and reduce HRV, vascular elasticity, and dilatation capacity (Brook et al., 2013; Kim and Hong, 2012; Schneider et al., 2008; Yeatts et al., 2007). Recently, novel indices that include PWA parameters and the RHI have been introduced to assess the impact of air pollution on endothelial function. These newer indices better identify all involved pathological pathways (Brook et al., 2016; Sun et al., 2013; Zhao et al., 2014).

These reports provided preliminary insights into the aforementioned two questions, but had certain limitations for an in-depth understanding of how air pollution affects individuals with cardiometabolic dysfunction. First, most studies were cross-sectional or observational in nature, rendering it difficult to draw causal inferences or to explore the dynamic impacts of air pollution on changes in metabolic traits. Furthermore, no study was designed to compare directly the effects of pollutants in healthy and cardiometabolically compromised individuals. Thirdly, earlier studies used limited biomarkers of certain biological pathways as health endpoints, therefore variations in cardiometabolic status could not be thoroughly assessed. Finally, current knowledge is inadequate for precise quantification of the health effects of individual $PM_{2.5}$ chemical and size fractional components.

A panel study is ideal for addressing the above limitations because it is versatile in multiple aspects. First, a panel study is deemed as an intensive prospective cohort study, repetitive measurements on each subject allow individual differences to be eliminated; this is impossible in cross-sectional and case-control studies. Second, matching subjects with cardiometabolic disorders to healthy controls enable us to better investigate population susceptibility. Additionally, panel study was conducted over a relatively short time, thus providing potential of obtaining abundant health endpoints and detailed exposure measurements. This is particularly useful when exploring the mechanism by which air pollution exerts acute effects on human health, and identifying the determinants of toxicity attributable to air pollution.

In this manuscript, we elaborate our design of a prospective panel study comparing the cardiometabolic and respiratory effects of air pollution exposure on healthy and pre-diabetic individuals (SCOPE) as a solution of the aforementioned questions. The results and limitations of previous studies are also summarized and compared with our study. Based on our accumulated experience, comprehensive evaluation was made to both health endpoints and exposure. Biomarkers at functional, cellular, and molecular levels were applied to reveal multiple biological pathways, including those we investigated in previous panel studies such as respiratory and cardiovascular inflammation (Han et al., 2016; Lin et al., 2011), oxidative stress (Lin et al., 2015), autonomic dysfunction (Sun et al., 2015), thrombosis (Gong et al., 2014; Rich et al., 2012), and those related to T2D progression such as insulin resistance and endothelial function. Metabolomics, as a powerful and rapidly developing technique that aids in the exploration of adverse effects on metabolic pathways (Yin et al., 2015), was also conducted in SCOPE. Taking advantage of a fixed site for air pollution monitoring, we obtained the concentration of multiple pollutants, including the detailed components of particles, such as size fractions, carbonaceous and metal compositions. Personal monitors will also be applied in this study to improve the accuracy of exposure assessment.

The primary aims of SCOPE were (i) to examine whether short-term exposure to air pollution adversely affected cardiometabolic and respiratory biomarkers, especially those related to T2D development; (ii) to determine whether individuals with cardiometabolic dysfunctions were more susceptible than healthy controls to air pollution, and the underlying mechanisms of such susceptibility; (iii) to quantify the health effects of individual air pollutants including different chemical and size-segregated particles.

RESEARCH DESIGN AND METHODS

Study participants and recruitment

All subjects recruited for the SCOPE study lived near the Peking University (PKU) campus and underwent annual physical examinations in PKU Hospital during 2013. Among a total of 22,343 participants of annual physical examinations, we recruited 60 pre-diabetic subjects (preDMs) and 60 healthy subjects. PreDMs were defined only by a fasting plasma glucose level of 6.1–7.0 mmol L^{-1} (i.e., not by impaired glucose tolerance) measured at the latest annual physical examination. The reason for choosing prediabetes instead of diabetes as case group is that preDM are individuals in the physiopathology state of cardiometabolic disorder, prone to have diabetes, but meanwhile do not need to take any hypoglycemic medicines which may bias the effect of air pollution on the glucose and lipid metabolic biomarkers. We also controlled for variables such as smoking that might bias the assessment of exposure to pollutants, and for the presence of certain diseases (Table 1).

Recruitment was based on the results of the annual physical examinations, telephone conversations, and responses to advertisements posted in the hospital. We conducted a chart screening among the participants of annual physical examination following the inclusion and exclusion criteria stated in Table 1, and the numbers of eligible subjects during step by step screening were shown in Figure S1 in Supporting Information. Potential subjects who met the recruitment criteria were selected randomly and were contacted by phone to confirm the willingness of participation. Additionally, 2 pre-diabetic and 8 healthy subjects were recruited by advertising poster in the hospital. Subjects were mainly faculties and staffs of Peking University, and certain amount of subjects were clustered in staff residential communities around PKU campus (Table S1 in Supporting Information). There were 81 (67.5%) subjects living within 5 km from the fixed monitoring site located in PKU campus, and 4 (0.3%) subjects living outside 10 km. Detailed description of subjects residential location was shown in Figure S2 in Supporting Information. Eligible subjects were educated on the study protocol and completed baseline questionnaires regarding their income, dietary habits, occupational exposure to pollutants, and medication use. The study protocol was approved by the Institutional Review Board of the PKU Health Science Center, and written informed consent was obtained from all subjects prior to study commencement.

The baseline demographic information of our study subjects were listed in Table 2. We compared the demographic structure of preDM and healthy individuals, and represented *P* value of the *t*-test and chi-square test results for numerical variables and classified variables, respectively. In all, female were slightly more than male subjects (69:51). There was no significant difference of age, height, weight, and gender ratio between preDM and healthy subjects. However, healthy subjects showed slightly higher educational level than preDM subjects (P=0.06), where 82% subjects finished college education in healthy group compared to 65% in preDM group. Eight subjects (5 preDM subjects and 3 healthy subjects) were found as current smoker later during the clinical visits. We took smoking status as covariate in the further data analysis rather than excluding these smokers considering they have already completed several clinical examinations.

Study protocol

Previous epidemiological studies have measured the acute health effects of air pollution based on pollutant exposure within the prior 2 weeks (Brook et al., 2010). Therefore, we commenced fixed-site monitoring 2 weeks prior to the clinical visits. Each subject attended four clinical sessions in different seasons within 1 year (Figure 1). Exposure to air pollutants was assessed in three ways, details description could be found in the following section and Table 3, in brief: (i) by online monitoring of pollution at PKU (the concentrations of common ambient air pollutants over the preceding 1–14 days were monitored at high-level time resolution); (ii) by offline analysis of daily filter samples (comprehensive analyses of particulate matter present on each of the preceding 14 days); and (iii) by analysis of filters from personal samplers worn over the 24 h prior to the clinical visit, which allowed for accurate evaluation of $PM_{2.5}$ levels and other toxic com-

Table 1 Recruitment criteria

Inclusion
1 PreDM subjects (6.1<FBG<7.0 mmol) Healthy subjects (FBG<6.1 mmol)
2 50<age<65
3 Nonsmoker or those who has quitted smoking longer than 3 years
Exclusion
1 Diagnosed with diabetes
2 Family diabetes history
3 History of any CV or metabolic diseases, including hypertension, hyperlipidemia, tumor, coronary heart disease, cardiomyopathy, arrhythmia, stroke, hepatitis A/B, leukemia, biliary calculus, thyroid nodule, sick sinus syndrome or wearing cardiac pacemaker, hyperthyroidism, hypothyroidism, multiple myeloma, rheumatoid, pancreatitis, reflux esophagitis, thyroidectomy

Table 2 Demographic information of study subjects

	Total	Pre-DM	Health	*P* value
N	120	60	60	
Sex				
Male	51 (43%)	24 (40%)	27 (45%)	0.71
Female	69 (58%)	36 (60%)	33 (55%)	
Age	57.0±4.3	57.7±4.2	56.4±4.4	0.10
Height	165.2±8.2	164.3±8.7	166.1±7.6	0.24
Weight	67.0±11.2	67.2±10.6	66.8±11.9	0.86
BMI	24.5±3.4	24.9±3.3	24.2±3.5	0.25
Educational level				
Middle school and below	32 (27%)	21 (35%)	11 (18%)	0.06
College and above	88 (73%)	39 (65%)	49 (82%)	
Work status				
Working	73 (61%)	33 (55%)	40 (67%)	0.26
Retired	47 (39%)	27 (45%)	20 (33%)	

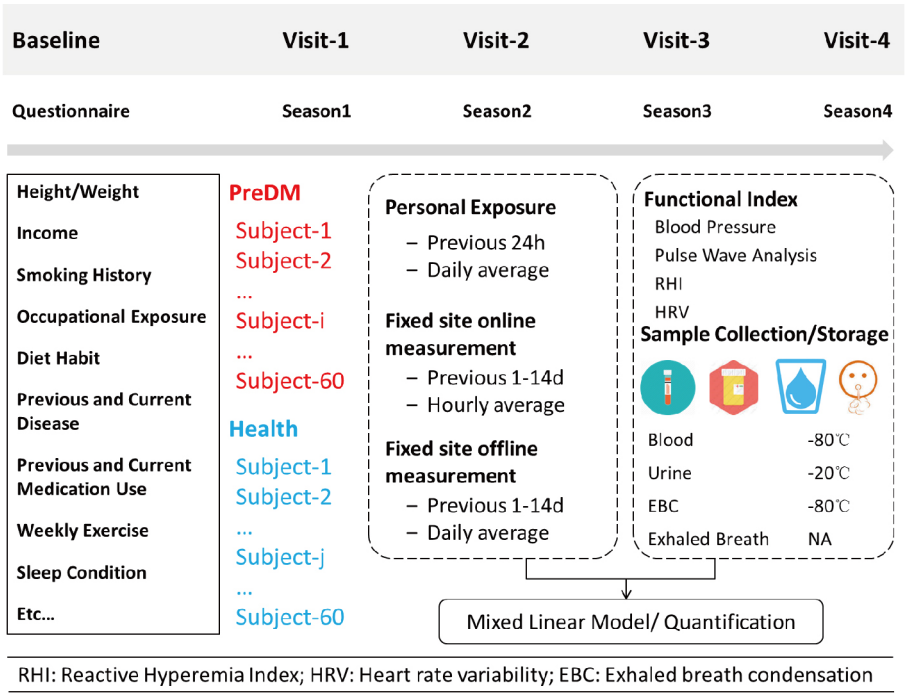

Figure 1 (Color online) Schemes of SCOPE study (repeated follow-up visits within one year).

ponents. On the day of the clinical visit, the personal sampler was given back to the investigator. Next, subjects completed a short questionnaire on sleep habits, pollution exposure, and medication used over the previous 3 days; underwent multiple functional examinations; and gave biological samples. Each visit lasted for less than 1 h. The details of exposure assessment and the health evaluations performed are described below.

Exposure assessment

Fixed-site measurements

Ambient pollutants were measured on the roof of a six-floor building of PKU (Guo et al., 2014). $PM_{2.5}$ concentrations were measured hourly using a tapered-element oscillating microbalance (TEOM; RP1400a instrument; Thermo Scientific, USA). Gaseous pollutants (CO, NO_x, SO_2, and O_3) were measured with online analyzers (models 48i, 42i, 43c, and 49i, respectively, Thermo Scientific). The numbers of size-segregated particles of aerodynamic diameters 5.6–560 nm were monitored with Fast Mobility Particle Sizer (FMPS Model 3091, TSI Inc., USA). Mass concentration of black carbon (BC) was measured using a Multi-Angle Absorption Photometer (MAAP Model 5012, Thermo Scientific). Meteorological variables including temperature, relative humidity

Table 3 The matrix of exposure parameters in SCOPE study

Exposure index	Parameters	Method/Instrument	Resolution
Particulate pollutants	$PM_{2.5}$ mass concentration	TEOM/Thermo RP1400a	Hourly
	BC mass concentration	MAAP/Thermo Model 5012	Hourly
	Size distribution (D=5.6–560 nm)	FMPS/TSI 3091	Hourly
	EC/OC	Sunset	Daily
	Water soluble ions Water soluble organic acid	Dionex ICS-2500/2000 Liquid Chromatogram	Daily
	Metal element	Thermo X series ICP-MS	Daily
	PAHs	Agilent GC-MS	Daily
Gaseous pollutants	CO	NDIR/Thermo Model 48i	Hourly
	NO_x	Chemiluminescence/Thermo Model 42i	Hourly
	SO_2	Fluorescence/Thermo Model 43c	Hourly
	O_3	UV absorption/Thermo Model 49i	Hourly
Meteorological parameters	Temperature, relative humidity, barometric pressure, wind speed, wind direction	Met one	Hourly

(RH), barometric pressure, and wind speed and direction were measured with Met One (Thermo Scientific). All instruments were calibrated and serviced every week.

During the periods of clinical visits, Teflon and quartz $PM_{2.5}$ filter samples were collected daily using a four-channel monitor, and the levels of elemental carbon (EC), organic carbon (OC), SO_4^{2-}, NO_3^-, NH_4^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, F^-, Cl^-, water-soluble organic compounds (WSOCs; including formic acid, methane sulfonic acid, acetic acid, and oxalic acid), and polycyclic aromatic hydrocarbons (PAHs) were later measured in the laboratory (Lin et al., 2015). Sample collection commenced at 08:00 and ended almost 24 h later, at 07:30 on the day of the clinical visit, thus reflecting pollutant exposure on the day before the visit.

Personal exposure

We used personal samplers recommended by the Environmental Protection Agency (Libra Model L-4, A.P. Buck, Inc., USA) equipped with a $PM_{2.5}$ cutoff inlet and a 37-mm quartz filter to obtain the mass concentrations of $PM_{2.5}$, EC, OC, and PAHs (Lin et al., 2015). The quartz filters were prepared in a muffle furnace held at 550°C for 5.5 h to eliminate background OC. All filters were weighed after placing in a superclean lab with a constant temperature and humidity (25°C, 40%) for at least 24 h. We calibrated the flow of each personal sampler to 4 L min^{-1}. Each subject was instructed to turn the sampler on at 24 h before the scheduled clinical visit, to carry the sampler when outdoors, and to put the sampler nearby when performing indoor activities such as cooking and sleeping.

Clinical visits

All the health endpoints and related biological pathways were listed in Table 4. The description in this section will follow the order of functional measurements, biosamples collection and cellular and molecular biomarkers analysis.

Functional health endpoints

To minimize systemic errors, measurements of all functional health endpoints were performed by the same trained staff throughout the study.

Blood pressure. The brachial artery blood pressure (both systolic (SBP) and diastolic (DBP)) was manually measured with a mercury sphygmomanometer prior to all other vascular measurements. Each subject was asked to rest quietly for at least 5 min, and then, while sitting, to extend the dominant arm onto a flat surface, ensuring that the elbow was at heart level.

Pulse wave analysis (PWA). PWA indices were noninvasively measured using a SphygmoCor PxPulse Wave Analysis System (SphygmoCor; AtCor Medical, Australia). We placed a high-fidelity micromanometer on the right wrist of each subject, over the radial artery; the device automatically flattened the artery and selected an optimal sensing element for recording the pulse wave. The pressure waveforms were calibrated by reference to the peripheral blood pressure using a built-in algorithm.

The quality of each pressure waveform was validated using a system operation index that evaluated waveform homogeneity (average pulse height, pulse height variation, shape deviation, and diastolic variation). Measurements with operation indices >85 (of a possible 100) were considered acceptable.

The PWA yielded several key indices of endothelial and heart function: central aortic blood pressure, augmentation pressure (AP), the augmentation index (AIx), ejection duration (ED), and the subendocardial viability ratio (SEVR).

Central aortic blood pressure was calculated using the

Table 4 The matrix of health endpoints in SCOPE study

Biological pathways	Sample/Device	Health endpoints
Blood pressure and endothelial function	Mercurial sphygmomanometer	Systolic pressure/Diastolic pressure
	SphygmoCor	PWA: AP/AP75/AIx/AIx75/ED/SEVR
	EndoPat 2000	RHI
HRV	EndoPat 2000	SDNN/RMSSD/PNN50/HF/LF/HR
Respiratory inflammation	Exhaled breath	FE_{NO}
	EBC	pH
		IL1α/IL1β/IL2/IL6/IL8/TNF-α/IFN-γ
Cardiovascular inflammation	Serum	CRP
		IL1α/IL1β/IL2/IL6/IL8/TNF-α/IFN-γ
	Plasma	WBC/neutrophil/monocyte/lymphocyte
Cardiometabolic index	Serum	TG/HDL/LDL/cholesterol
		Glucose/Insulin/HOMA-IR
	Plasma	HbA1c
Oxidative stress	Urine	MDA/Creatinine

brachial artery blood pressure and the recorded pulse waveforms. AP was calculated as the maximum central aortic systolic pressure minus the pressure at the inflection point, and AIx as the ratio of AP to pulse pressure (expressed as a percentage). Higher AP and AIx values indicate increased wave reflection from the periphery, or earlier return of the reflected wave, attributable to arterial stiffness or vascular resistance (Weber et al., 2004). As the ascending aortic pressure is strongly dependent on the heart rate, the AP and AIx values were normalized to a heart rate of 75 beats per min (expressed as AP75 and AIx75) to control for individual differences. The ED was determined from the beginning of a pulse to the time of the dicrotic notch of the radial tonometric pulse. SEVR was calculated as the ratio of the diastolic to the systolic tension time index, determined using ED. ED and SEVR reflect the extent of subendocardial myocardial perfusion (Chemla et al., 2008).

Reactive hyperemia index (RHI). RHI was assessed using a non-invasive plethysmographic instrument (EndoPAT2000; Itamar Medical, Ltd., Israel), which measures the pulsatile blood volume in the fingertips of both hands. A blood pressure cuff was placed on the non-dominant arm and inflated to 50 mmHg greater than the systolic pressure for 5 min to induce brachial arterial occlusion, and then released to induce reactive hyperemia. The contralateral arm served as a control. Two finger-mounted probes were placed on the index fingers to detect pulsatile volume changes during equilibration, occlusion, and hyperemia. The RHI was calculated as the ratio of the average pulse wave amplitude during hyperemia to the average baseline amplitude of the occluded arm, divided by the same values of the control arm, and then multiplied by a baseline correction factor (Hamburg et al., 2008). RHI is an indicator of microvascular endothelial function, which is pathologically compromised early in the atherosclerotic process (Gandhi and Rao, 2014).

Heart rate variability (HRV). HRV data were obtained by the EndoPAT 2000 during the 5.5-min baseline equilibration period of endothelial function assessment. Each measure was based on the criteria of the European Society of Cardiology and the North American Society of Pacing Electrophysiology Task Force (Gandhi and Rao, 2014). HRV was analyzed in both the time and frequency domains. Time-domain analysis was based on evaluation of beat-to-beat changes in the heart rate, as revealed by the sinus R-R intervals over time, and generated the standard deviation of the N-N interval (SDNN), the root mean square of successive differences (RMSSD), the number of pairs of successive N-Ns that differed by more than 50 ms (NN50s), and the number of NN50s divided by the total number of N-Ns (pNN50). Frequency-domain analysis evaluated the spectrum density of R-R intervals within specific frequency bands, including low frequency (LF) (0.04–0.15 Hz) and high frequency (HF) (0.15–0.40 Hz).

Biological samples

Exhaled breath. We collected exhaled breath following the protocol recommended by the American Thoracic Society and the European Respiratory Society. Briefly, each subject was instructed to rinse the mouth, inhale to tidal capacity, and exhale into a 4-L aluminum air-sampling bag at a constant flow rate of 150 mL s^{-1}. A detailed description of such sampling and the associated quality control was given in a previous report (Lin et al., 2011).

Exhaled breath condensate. Exhaled breath condensate (EBC) was collected using a Jaeger EcoScreen collector (Erich Jaeger, Germany). The machine was switched on at least 30 min before collection to allow the cooling cuff to stabilize at an operating temperature of –20°C. Each subject was asked to wear a nose clip and calmly breathe

into the condenser pipe for 10 min; the exhaled breath condensed at the low temperature and was collected as a liquid (Barraza-Villarreal et al., 2008). EBC samples were stored in centrifuge tubes at –80°C prior to cytokine analysis.

Blood samples. Blood samples were collected by nurse before 09:30 during the clinical visits to avoid the risk of hypoglycemia. Subjects were asked to fast overnight (for at least 12 h) to minimize extraneous effects on platelet activation. Plasma samples were stored in EDTA-coated tubes, and immediately placed in an ice-filled box. Serum samples were stored in pro-coagulation tubes and placed in the ice-filled box after 30 min of clotting at room temperature. Some biomarkers (please see below) were measured in the clinic immediately after sample collection. Residual samples were aliquoted within 2 h of 4°C placement and stored at –80°C prior to cytokine and metabolite analyses.

Urine samples. First-morning urine samples were used to evaluate oxidative stress and the levels of certain metabolites. Each subject collected a midstream urine sample into a 15-mL polypropylene tube after waking and brought it to the clinic. All samples were stored in centrifuge tubes at –20°C.

Biomarker analysis

FE_{NO}. The nitric oxide concentration in exhaled breath (FE_{NO}) is a recommended surrogate clinical biomarker of acute subclinical airway inflammation. NO concentrations in sampling bags were measured within 4 h using a calibrated chemiluminescence nitrogen oxide analyzer (model 42i; Thermo Scientific) (Han et al., 2016; Lin et al., 2011).

EBC pH. The pH of the EBC sample is a robust and reproducible indicator of airway acidity (Vaughan et al., 2003). The pH was measured (InLab Micro; Metler Toledo, Swiserland) immediately after EBC sample collection and again after 5 min of de-aeration with inert argon.

Cardiometabolic index. To explore cardiometabolic status, indices of glucose and lipid metabolism were automatically measured in the hospital immediately after sample collection. Specifically, we measured fasting glucose levels and the levels of blood lipids (triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and total cholesterol) using an Olympus AU2700 biochemical analyzer; and the level of glycosylated hemoglobin (HbA1c) employing a Bio-Rad Variant II Turbo analyzer. HOMA-IR (an index based on a homeostatic model; the product of the levels of fasting glucose and insulin) was calculated to estimate basal insulin sensitivity and indicate insulin resistance.

Blood cell counts and the C-reactive protein (CRP) level. Whole blood in EDTA-coated tubes was sent to the clinic for analysis of 31 parameters by a Sysmex XE-2100 Automated Hematology System, including the concentrations of white blood cells (WBCs), neutrophils, monocytes, lymphocytes, red blood cells, hemoglobin, and platelets. CRP levels in plasma samples were measured via automated nephelometry.

Cytokines in EBC and serum. Exposure to air pollution is associated with increased levels of multiple cytokines including IL1α, IL1β, IL2, LI6, IL8, TNF-α, and IFN-γ (Calderón-Garcidueñas et al., 2013; Ouyang et al., 2000). We simultaneously assessed respiratory and systemic inflammation by measuring the levels of multiple cytokines in both EBC and serum samples using the Cytometric Bead Array (CBA) method and a BD FACSCalibur flow cytometer (Becton Dickinson, USA) (Li et al., 2016).

The EBC and serum samples were first appropriately diluted. Capture beads bearing specific antibodies were suspended in diluent and thoroughly mixed with the samples to allow cytokine binding. After 1 h of incubation at room temperature, phycoerythrin (PE)-labeled antibodies were dissolved in the detection reagent diluent and added to the sample tubes, then left to stand at room temperature in the dark for 1.5 h to allow the fluorescent antibodies to fully bind. The mixtures were washed with buffer and centrifuged, and the resulting pellets resuspended in detection reagent, followed by BD FACSCalibur flow cytometry and analysis using FCAP Array version 3.0 software. The cytometer was calibrated before each analysis using reference beads provided by the manufacturer.

Oxidative stress biomarkers in urine samples. We measured urinary malondialdehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHdG) to assess the oxidative stress levels. MDA is a final product of lipid peroxidation generated when reactive oxygen species attack the cell membrane. MDA can attach to certain proteins including LDL, compromising their functioning (Suzuki et al., 2010). 8-OHdG is one of the predominant products of free radical-induced lesions of DNA, which can exist stably in urine samples (Wu et al., 2004). Detailed description of the analyses of MDA and 8-OHdG was reported in our previous study (Lin et al., 2015). Briefly, urinary MDA concentrations were measured by reaction with 2-thiobarbituric acid (TBA), followed by high-performance liquid chromatography (Waters 2695), with monitoring of absorption at 532 nm. For 8-OHdG, we used solid phase extraction method to remove interfering substance in urine sample and then measured with HPLC and electrochemical detector (ECD, Waters 2465). Urinary creatinine were also measured spectrometrically at 510 nm to adjust the levels of urinary MDA and 8-OHdG.

QA/QC. All of the instruments used for the biomarker analysis were calibrated regularly. For example, the NO analyzer was calibrated at the beginning of the campaign with five concentrations among the span range and the coefficient of determination (R^2) was above 0.98. Zero check was then performed every week. The pH meter was calibrated every week with standard buffers (pH=4.01, 7.0 and 9.21) in room temperature, and the electrode was stored in 3 mol L^{-1} KCl solution after carefully rinsed by DI water. Biochemical analyzer and hematology system were calibrated every day by

the clinical laboratory.

Metabolomic analysis

Plasma samples were subjected to non-targeting high-throughput metabolomic analysis via gas chromatography-mass spectrometry (GC/MS) and liquid chromatography-mass spectrometry (LC/MS). Several blood pretreatments have been reported; the principal features of all are deproteinization and extraction (Yin et al., 2015). We applied a simple widely accepted method ensuring adequate coverage of metabolites (Dunn et al., 2011). Methanol with the volume three times of the plasma sample was added to each plasma sample at room temperature (15°C–20°C) to precipitate proteins. Supernatants were collected after centrifugation at 10,000 r min^{-1} for 10 min at 4°C and lyophilized prior to either chemical derivatization (GC-MS; Agilent 7200 Q-TOF system) or reconstitution in 50% (v/v) methanol (LC/MS; Agilent 6550 iFunnel Q-TOF System, USA). MassHunter Workstation Software was used for both qualitative and quantitative analyses of the raw dataset. Detailed methods are still in the process of optimization.

Statistical analyses

Online data were grouped by hourly and daily means. Consistent with the timing of clinical visits, daily exposures were calculated as the 24-h averages commencing at 08:00 on each day, and were considered invalid if >8 h of data were missing for any day. All health outcome variables were subjected to normality testing, and those that failed the test were log-transformed prior to regression modeling.

A linear mixed model was used to assess within-subject correlations among repeated outcome measures, and fixed and random effects were estimated employing the restricted maximum likelihood method. Age, body mass index (BMI), sex, and cardiometabolic status (PreDM vs. Healthy) were added to the model as adjusted covariants. To capture the potential nonlinear effects of confounders such as temperature, relative humidity, and the day of the week (DOW), we used natural spline functions with up to three degrees of freedom, and determined model parameters by reference to the Akaike information criterion. The final equation was:

$$\begin{aligned} E(Y_{ij}|X_{ij}) = {} & \alpha_0 + \beta_1 \cdot X_{ij} + f_{ns}(\mathrm{Temp}_{24\,\mathrm{h}ij}, \mathrm{d}f) \\ & + f_{ns}(\mathrm{RH}_{24\mathrm{h}ij}, \mathrm{d}f) + f_{ns}(\mathrm{Temp}_{7\,\mathrm{d}ij}, \mathrm{d}f) \\ & + f_{ns}(\mathrm{Temp}_{7\,\mathrm{d}ij}, \mathrm{d}f) + f_{ns}(\mathrm{DOW}_{ij}, \mathrm{d}f) \\ & + \beta_2 \cdot \mathrm{Age}_{ij} + \beta_3 \cdot \mathrm{BMI}_{ij} + \beta_4 \cdot \mathrm{Sex}_{ij} + \varepsilon_{ij} \end{aligned}$$

where i refers to a subject, j refers to a visit, X_{ij} and Y_{ij} are the level of exposure to a pollutant and the biomarker concentrations of subject i measured at visit j, f_{ns} is the natural spline function, df is the degree(s) of freedom, and DOW the day of the week.

We firstly examined associations by pairing every individual air pollutant with all health endpoints using a linear mixed model. Health effects were estimated by changes in biomarker responses per interquartile increase in each pollutant. To identify cumulative effects, pollutant concentrations were averaged over various time intervals (including 1–24 h and 1–14 days) prior to health outcome measurements. Furthermore, we included interaction term between exposure and the cardiometabolic status when evaluating the responses of preDM subjects and healthy controls, and we calculated the significance levels of variations in effects between the two groups.

DISCUSSION

The SCOPE study was designed to investigate how air pollution affects individuals with cardiometabolic dysfunction and the underlying mechanisms especially related to T2D development, and examine if cardiometabolic disorders increased susceptibility to air pollution. In this manuscript, we elaborate the design and underlying considerations of SCOPE study. Extensive results and discussion will be reported in the papers that following this one. Our findings may be of considerable public health importance given the nationwide diabetes epidemic and severe air pollution in China.

Although growing evidence indicates that air pollution affects multiple pathways involved in metabolic syndrome, previous studies have featured different populations (children, adults, elderly individuals, patients with asthma, and diabetic patients) and focused on only a few pathways; this may render the conclusions inconsistent. Therefore, we evaluated multiple biological pathways in a targeted group (preDM subjects and healthy controls) to systematically explore the effects of air pollution.

Disorders of glycolipid metabolism/insulin resistance, endothelial dysfunction, and systematic inflammation are the three major causes of T2D exacerbation (Chuang et al., 2011; Kelishadi et al., 2009; O'Neill et al., 2007; Schneider et al., 2008). We evaluated a range of health endpoints at the functional, cellular, and molecular levels, covering all relevant mechanistic pathways. For example, we measured (i) fasting glucose, insulin, triglyceride, total cholesterol levels, and HOMA-IR to assess glycolipid metabolic status; (ii) blood pressure, PWA, and RHI to evaluate endothelial function; and (iii) WBC, CRP, and cytokine levels in both EBC and serum to assess the status of respiratory and cardiovascular inflammation.

Previous studies did not adequately address whether those with impaired glucose metabolism were susceptible to air pollution. The evidence were primarily based on ecological or cross sectional studies which suggested that T2D might increase the risk of PM-associated hospital admission for

cardiovascular problems, mortality (Goldberg et al., 2006; Zanobetti and Schwartz, 2002), and susceptibility to impaired endothelial function (O'Neill et al., 2005). This prospective panel study examined the development of changes in both preDM patients and healthy controls may fill an obvious gap.

The responses of preDM and healthy individuals might differ greatly for several reasons. First, differences may appear in certain pathways and in target organs, based on the evidence from animal experiments focused on particle-induced cardiometabolic and respiratory health endpoints. For instance, mice on a high-fat diet, compared to those receiving a normal diet, are more likely to develop atherosclerosis when exposing to $PM_{2.5}$ (Sun et al., 2005). An *in vivo* study using alveolar macrophages from diabetic rabbits indicated that cardiometabolic disease might enhance respiratory system inflammation (Mo et al., 2009). We also observed in our previous panel study that FE_{NO} levels were significantly higher in T2D than in preDM subjects (Han et al., 2016). Second, preDM subjects and healthy controls may respond differently to particles of certain sizes formed of varying components. Compared to larger particles, smaller particles were concentrated at higher numbers, were more efficiently deposited in the respiratory tract, had larger active surface areas, and tended to absorb more toxic chemicals, such as organics (Oberdörster et al., 2005). Some organic chemicals, including persistent organic pollutants, have been shown to enhance T2D development (Rignell-Hydbom et al., 2009). Furthermore, differences may also reflect in the lag effect between the two groups. PreDM patients are in the early stages of glucose levels and endothelial dysfunction impairment, and exhibited increased blood vessel permeability and a lower capacity to control glucose metabolism. Such basic pathological features may trigger stronger, earlier, and longer-lasting responses to air pollution. These potential differences are considered in advance and are sufficient to be investigated based on the current design of SCOPE study.

The SCOPE study had a number of useful strengths; we (i) prospectively explored the associations between air pollution and cardiometabolic disease in humans; (ii) determined whether cardiometabolic disorders increased susceptibility to air pollution by comparing the effects in the two groups; (iii) evaluated systemic and dynamic changes in cardiometabolic and respiratory responses by including numbers of functional, cellular, and molecular health endpoints, which together may allow determination of the underlying biological mechanisms of DM progression and susceptibility; (iv) comprehensively evaluated the levels of ambient air pollutants, including particles of different sizes and chemical compositions, targeting the critical toxic suspects among the large matrix of pollutants; (v) used filter-based personal monitors to accurately record exposures to certain PM components, including transition metals and carbonaceous components, and to better control of the effects of between-subject variability; and (vi) constructed a matrix of biomarkers and employed metabolomics to comprehensively evaluate health status and responses to air pollution. Notably, metabolomics, which is both comprehensive and unbiased, facilitated the discovery of new biomarkers and revealed novel mechanisms of action.

This study has some limitation. First, our personal exposure assessments were limited, especially in term of duration. As wearing a personal monitor is burdensome, we collected lag data for only 1 day. However, we measured many particulate chemicals in personal filters, and we obtained lag data of longer duration employing fixed-site monitoring. Second, all subjects were aged 50–65 years and were of relatively high socioeconomic status. Therefore, our findings may not be generalizable to those aged >65 years and those of lower socioeconomic status, who may be more susceptible to air pollution. Third, we measured many air pollutants simultaneously, and this was possible for only short periods. Thus, we focused on the acute effects of air pollution within the exposure duration of 2 weeks. Long-term effects of air pollution could be an influential factor during one year study period, and panel study design is limited in capturing the changes of chronic cardiometabolic disease such as diabetes. A future long-term study could be useful to examine T2D exacerbation caused by air pollutants. Finally, although our sample size was relatively large compared to those of typical panel studies, the size may not have been adequate to allow us to recognize mild effects of pollution with marginal significance on certain biomarkers.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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SUPPORTING INFORMATION

Figure S1 Step by step screening strategy.

Figure S2 Description of subjects residential location.

Table S1 Community clustering condition

The supporting information is available online at http://life.scichina.com and https://link.springer.com. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.