The Effect of Urban Air Pollution on Inflammation, Oxidative Stress, Coagulation, and Autonomic Dysfunction in Young Adults

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***Rationale*: The biological mechanisms linking air pollution to cardiovascular events still remain largely unclear.**

***Objectives*: To investigate whether biological mechanisms linking air pollution to cardiovascular events occurred concurrently in human subjects exposed to urban air pollutants.**

***Methods*: We recruited a panel of 76 young, healthy students from a university in Taipei. Between April and June of 2004 or 2005, three measurements were made in each participant of high-sensitivity C-reactive protein (hs-CRP), 8-hydroxy-2′-deoxyguanosine (8-OHdG), plasminogen activator fibrinogen inhibitor-1 (PAI-1), tissue-type plasminogen activator (tPA) in plasma, and heart rate variability (HRV). Gaseous air pollutants were measured at one air-monitoring station inside their campus, and particulate air pollutants were measured at one particulate matter supersite monitoring station 1 km from their campus. We used linear mixed-effects models to associate biological endpoints with individual air pollutants averaged over 1- to 3-day periods before measurements were performed.**

***Measurements and Main Results*: We found that increases in hs-CRP, 8-OHdG, fibrinogen, and PAI-1, and decreases in HRV indices were associated with increases in levels of particles with aerodynamic diameters less than 10 μm and 2.5 μm, sulfate, nitrate, and ozone (O_3) in single-pollutant models. The increase in 8-OHdG, fibrinogen, and PAI-1, and the reduction in HRV remained significantly associated with 3-day averaged sulfate and O_3 levels in two-pollutant models. There were moderate correlations ($r = -0.3$) between blood markers of hs-CRP, fibrinogen, PAI-1, and HRV indices.**

***Conclusions*: Urban air pollution is associated with inflammation, oxidative stress, blood coagulation and autonomic dysfunction simultaneously in healthy young humans, with sulfate and O_3 as two major traffic-related pollutants contributing to such effects.**

Keywords: air pollution; inflammation; oxidative stress; blood coagulation; heart rate variability

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

The mechanisms linking air pollution to cardiovascular diseases involve direct effects of air pollution on the lung and cardiovascular system and indirect effects mediated through pulmonary inflammation and oxidative stress.

What This Study Adds to the Field

Urban air pollution is associated with inflammation, oxidative stress, blood coagulation and autonomic dysfunction simultaneously in healthy young humans, with sulfate and O_3 as two major traffic-related pollutants contributing to such effects.

The epidemiological association between exposure to air pollution and cardiovascular morbidity and mortality has been well documented in previous studies (1, 2). The underlying mechanisms linking pulmonary exposure to air pollution with increased risk for cardiovascular events have also been investigated in the last decade. Recently, the American Heart Association (AHA) published a statement on the importance of air pollution in the development of cardiovascular disease. The potential biological mechanisms linking air pollution to cardiovascular diseases in the AHA statement involve direct effects of air pollution on the lung, and subsequently the cardiovascular system, through a neural mechanism to alter central nervous system functions or systemic circulation to directly interact with target tissues, and/or indirect effects mediated through pulmonary inflammation and oxidative stress that develop into a systemic inflammatory response (3). Several panel studies are in support of these mechanisms in human subjects. Some of these studies have documented the association between autonomic dysfunction and air pollutants, such as particulate air pollutants of particles with aerodynamic diameters less than 10 μm (PM_{10}), particles with aerodynamic diameters less than 2.5 μm ($PM_{2.5}$) (4–12), ozone (O_3) (4), sulfur dioxide (SO_2) (8), or nitrogen dioxide (NO_2) (13). Some studies also found that air pollution was associated with activation of inflammatory/oxidative stress pathways (6, 14–18), an increase in plasma viscosity (19, 20), or impairment in endothelial function (21, 22). Although these studies have individually (4, 5, 8–22) or simultaneously (6, 7, 17) identified certain cardiovascular endpoints related to air pollution, few of them have studied the overall biological mechanisms simultaneously, including inflammation, oxidative stress, blood coagulation, and autonomic function in the cardiovascular system among healthy young humans.

In the past few years, our epidemiological studies have also found significant associations between $PM_{2.5}$ and emergency admissions for cardiovascular (23) and cerebrovascular diseases (24) in Taipei. Recently, our panel studies (9, 25) reported that PM exposures were associated with autonomic dysfunction in young, healthy adults and patients in Taipei. Our recent panel study found that urban air pollution could increase the levels of the coagulation factor plasma fibrinogen and the fibrinolytic factor plasminogen activator inhibitor-1 (PAI-1) in patients with or at risk for cardiovascular diseases (26). Furthermore, sulfate and organic carbon (OC) in particulate matter collected in Taipei were capable of inducing inflammation, oxidative stress, and

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endothelial dysfunction in our *in vivo* (27, 28) and *in vitro* (29) studies.

Basically, each of our previous studies supported a part of the biological mechanisms linking air pollution to cardiovascular diseases listed in the AHA's statement. Such findings led us to hypothesize that air pollution should be associated with all of these biological mechanisms in humans. To test this hypothesis, we conducted this panel study to investigate whether inflammation, oxidative stress, blood coagulation, and autonomic dysfunction in humans simultaneously respond to changes in urban air pollution levels.

METHODS

Participants

Our study participants consisted of 76 healthy college students aged 18 to 25 years, living in the dormitory of the Fu-Jen Catholic University in the Taipei metropolitan area. The selection criteria of study participants were as follows: no history of smoking; no medication that might affect cardiac rhythm; and no cardiovascular diseases, such as coronary artery disease, arrhythmia, hypertension, diabetes mellitus, and dyslipidemia. Eighty-five college students responded to our recurring advertisement in the campus bulletin board system; 76 of them (89%) met the criteria and were willing to participate in this study after our protocols had been explained. We collected blood samples and conducted electrocardiographic (ECG) monitoring on each participant for three times from April to June in the 2004 or 2005. For each participant, we collected one sample per month for 3 months—April, May, and June. The average time between visits was approximately 30 days. The sampling date and time were recorded for each participant to match with air pollution and weather data in different exposure periods. During the participants' first visits, age, sex, and medical history were recorded using a questionnaire; height and weight were measured using a standardized protocol and used to calculate body mass index (BMI). The ethics committee of the National Taiwan University Hospital approved this study. Informed consent was obtained from each participant before the study began.

Markers of Systemic Inflammation and Oxidative Stress

We took 10-ml fasting blood sample each time from each participant in the morning of the sampling day and kept the sample on ice before centrifugation. These blood samples were first centrifuged by a refrigerated centrifuge at 3,000 rpm for 15 minutes within 90 minutes of collection and then stored at −80°C before assay. The inflammatory marker, high-sensitivity C-reactive protein (hs-CRP), was determined in serum by a two-site chemiluminescent enzyme immunometric assay (IMMULITE hs-CRP; Diagnostic Products Corp., CA). The oxidative stress marker, oxidative DNA adduct 8-hydroxy-2′-deoxyguanosine (8-OHdG), was measured by enzyme-linked immunosorbent assay (ELISA) based on monoclonal antibody N45.1 (30) (Japan Institute for the Control of Aging, Fukuroi City, Japan).

Fibrinolytic and Coagulation Factors

Two fibrinolytic factors, plasma tissue-type plasminogen activator (tPA) and PAI-1, and one coagulation factor, fibrinogen, were measured in this study. Fibrinogen was measured by the clotting method of Clauss, using STA-Fibrinogen 5 kits (Diagnostica Stago, Taverny, France). Antigens of tPA and PAI-1 were measured by the ELISA method (Imubind tPA ELISA and Imubind PAI-1 ELISA; American Diagnostica, Greenwich, CT).

Heart Rate Variability Indices

After blood sample collection and a 15-minute rest for each participant, we immediately performed a 16-minute resting ECG, in the sitting position, during daytime (8:00 A.M. to 12:00 P.M.) using a PacerCorder three-channel device (model 461A; Del Mar Medical Systems LLC, Irvine, CA) with a sampling rate of 250 Hz (4 ms). ECG tapes were sent to National Taiwan University Hospital and processed using a Delmar Avionics model Strata Scan 563 (Irvine, CA). A complete 5-minute segment of NN interval was taken for heart rate variability (HRV) analysis, including standard deviation of NN intervals (SDNN), the square root of the mean of the sum of the squares of differences between adjacent NN intervals (r-MSSD), low frequency (LF) (0.04–0.15 Hz), and high frequency (HF) (0.15–0.40 Hz). The detailed description of translating ECG wave complexes (QRS) to HRV indices is given in our previous study (9).

Air Pollution and Weather Data

Fixed-site monitoring data were used to represent all study participants' exposures to air pollutants. Hourly concentrations of PM_{10}, O_3, SO_2, NO_2, and carbon monoxide (CO) were obtained from the air-monitoring station in the Fu-Jen campus. The instrumentation used in the fixed-site monitoring stations are β-gauge for PM_{10}; ultraviolet (UV) absorption for O_3; UV fluorescence for SO_2; chemiluminescence for NO_2 and nondispersed infrared absorption for CO. Hourly levels of $PM_{2.5}$; components of $PM_{2.5}$, including sulfate, nitrate, OC, and elemental carbon (EC); temperature; and relative humidity were obtained from one PM supersite monitoring station, located 1 km from the Fu-Jen campus. The collocated Rupprecht and Patashnick 1400a tapered element oscillating microbalance sampler (Rupprecht and Patashnick Co., Inc., Albany, NY) was used to determine the concentrations of $PM_{2.5}$. The ambient carbon particulate monitor 5400 (Rupprecht and Patashnick Co., Inc.) was used to determine the concentrations of organic and elemental carbon in $PM_{2.5}$. The ambient particulate nitrate monitor 8400N and the ambient particulate sulfate monitor 8400S (both, Rupprecht and Patashnick Co., Inc.) were used to determine the concentrations of nitrate and sulfate. All environmental data were matched with the sampling time of blood and HRV for each participant during their monitoring periods. The environmental data averaged by 24 hours (1-d average), 48 hours (2-d average), and 72 hours (3-d average) before the hour of each blood sampling were used to estimate pollution effects on blood markers and HRV indices.

Statistical Analysis

We used linear mixed-effects regression models in S-Plus 2000 (MathSoft, Inc., Cambridge, MA) to examine the association between individual air pollutants and blood markers and $\log_{10}$-transformed HRV. The exposure variables were 1- to 3-day averages of PM_{10}, $PM_{2.5}$, sulfate, nitrate, OC, EC, NO_2, CO, SO_2, and O_3, and the outcome variables were hs-CRP, fibrinogen, PAI-1, tPA, 8-OHdG, and $\log_{10}$-transformed HRV indices (SDNN, r-MSSD, LF, and HF). We treated participant's sex, age, and BMI, and weekday, temperature of the day before, relative humidity, and air pollution as fixed effects and fitted participants as a random intercept term in our mixed-effects models. Single-pollutant mixed-effects models were used to determine pollution effects for each pollutant separately. The air pollutants, which were significantly ($p < 0.05$) associated with outcome variables in the single-pollutant mixed-effects models, were considered for further analyses in multipollutant mixed-effects models.

Model selections were based on the criteria of minimizing Akaike's Information Criterion (31). Pollution effects are expressed as percent changes by interquartile range (IQR) changes as $[10^{(\beta \times \mathrm{IQR})} - 1] \times 100\%$ for $\log_{10}$-transformed HRV indices and $[\beta \times \mathrm{IQR} \div \mathrm{M}] \times 100\%$ for blood markers, where β and M are the estimated regression coefficient and the mean of each blood marker, respectively. The sensitivity analysis between HRV and air pollution, with adjustment for hs-CRP, fibrinogen, and PAI-1 separately, was further applied to the mixed-effects models to explore whether the association between HRV and air pollution is partially explained by certain blood markers.

RESULTS

Characteristics of Participants and Air Pollution

Our 76 participants' age averaged 20.8 years (SD = 1.2 yr), their BMI averaged 20.8 kg/m^2 (SD = 2.4 kg/m^2), and the male/female ratio was approximately 3:2. The mean (SD) values of blood markers for our study participants over their three visits were 296.6 mg/dl (54.2) for fibrinogen, 6.5 ng/ml (5.0) for tPA, 31.0 ng/ml (15.0) for PAI, 0.08 mg/dl (0.03) for hs-CRP, and 0.6 ng/ml (0.2) for 8-OHdG. The mean values (SD) of $\log_{10}$-HRV indices were 1.8 ms^2 (0.2) for SDNN, 1.2 ms^2 (0.2) for r-MSSD, 3.0 ms^2 (0.3) for LF, and 2.5 ms^2 (0.5) for HF.

Table 1 summarizes air pollution and meteorological data averaged over 1 to 3 days before blood sampling for the 76 study participants. During the study period, urban air pollution was comparatively high, especially with regard to PM_{10}, $PM_{2.5}$, PM components, and O_3, but the range in these pollutants was also relatively wide: for example, the 3-day averages ranged from 22.2 to 87.2 $\mu g/m^3$ for PM_{10}, 12.7 to 59.5 $\mu g/m^3$ for $PM_{2.5}$, and 22.5 to 48.3 ppb for O_3. The concentrations of certain PM components, such as nitrate, sulfate, and OC, were also concurrently elevated with PM: with 1- to 3-day averages being 4.4 to 4.7 $\mu g/m^3$ for nitrate, 3.9 to 4.1 $\mu g/m^3$ for sulfate, and 4.5 to 7.0 $\mu g/m^3$ for OC. The Pearson correlation analysis showed that $PM_{2.5}$ was significantly ($p < 0.001$) correlated with sulfate ($r = 0.9$), OC ($r = 0.8$), and EC ($r = 0.8$). The weather was moderately pleasant with a temperature range of 24.4 to 24.8°C and a relative humidity range of 68.6 to 69.6% during the study period.

TABLE 1. AIR POLLUTION LEVELS AND WEATHER CONDITIONS AT 1 TO 3 DAYS BEFORE MEASURING HEART RATE VARIABILITY AND COLLECTING BLOOD SAMPLES AMONG 76 STUDY SUBJECTS

Variable	1-d Average (*n = 228*)	2-d Average (*n = 228*)	3-d Average (*n = 228*)
PM_{10}, $\mu g/m^3$			
Mean ± SD	49.2 ± 18.0	55.3 ± 18.6	54.9 ± 18.2
IQR	32.7	34.5	26.0
Range	29.5–83.4	25.5–85.1	22.2–87.2
$PM_{2.5}$, $\mu g/m^3$			
Mean ± SD	31.8 ± 10.6	36.4 ± 12.6	36.5 ± 12.6
IQR	20.4	25.2	20.0
Range	16.2–50.1	15.0–53.4	12.7–59.5
Nitrate, $\mu g/m^3$			
Mean ± SD	4.5 ± 2.7	4.7 ± 2.4	4.4 ± 2.2
IQR	2.5	4.0	3.4
Range	0.7–10.6	0.7–8.9	0.8–7.5
Sulfate, $\mu g/m^3$			
Mean ± SD	4.1 ± 3.6	4.1 ± 3.7	3.9 ± 3.5
IQR	3.9	4.3	3.8
Range	0.4–10.9	0.4–11.9	0.4–11.5
OC, $\mu g/m^3$			
Mean ± SD	4.5 ± 3.1	6.6 ± 2.6	7.0 ± 3.3
IQR	1.0	2.3	2.0
Range	0.3–8.9	3.1–12.4	2.9–14.8
EC, $\mu g/m^3$			
Mean ± SD	1.6 ± 0.6	2.3 ± 1.5	3.3 ± 3.7
IQR	0.9	1.5	1.2
Range	0.4–2.4	0.8–5.3	1.0–11.7
O_3, ppb			
Mean ± SD	28.4 ± 12.1	33.3 ± 8.9	33.8 ± 7.1
IQR	17.9	16.0	12.0
Range	7.4–49.3	20.4–47.8	22.5–48.3
NO_2, ppb			
Mean ± SD	17.3 ± 14.7	16.5 ± 11.8	15.8 ± 11.5
IQR	19.6	13.6	17.0
Range	2.3–53.1	2.5–37.3	2.8–33.3
SO_2, ppb			
Mean ± SD	16.3 ± 11.9	18.5 ± 14.3	17.9 ± 13.7
IQR	22.4	27.9	27.5
Range	2.1–36.8	2.5–40.2	2.8–39.4
CO, ppm			
Mean ± SD	0.8 ± 0.4	0.8 ± 0.3	0.7 ± 0.3
IQR	0.5	0.4	0.4
Range	0.4–1.8	0.4–1.4	0.4–1.2
Temperature, °C			
Mean ± SD	24.8 ± 3.9	24.4 ± 3.9	24.4 ± 3.8
Range	17.3–29.7	17.0–29.3	17.1–29.1
Relative humidity, %			
Mean ± SD	69.6 ± 9.9	68.8 ± 8.1	68.6 ± 8.2
Range	55.6–86.7	56.6–80.9	55.4–81.4

Definition of abbreviations: EC = elemental carbon; IQR = interquartile range; OC = organic carbon.

Inflammatory, Oxidative Stress, Fibrinolytic, and Coagulation Factors

The pollution effects on inflammatory, oxidative stress, fibrinolytic, and coagulation factors estimated by single-pollutant mixed-effects models are listed in Table 2. The inflammatory marker hs-CRP was significantly associated with increases in PM_{10} at 1- and 3-day averages, sulfate at 1- to 3-day averages, and O_3 at 2- to 3-day averages. The oxidative stress marker 8-OHdG was significantly associated with increases in nitrate at 1- to 3-day averages, and sulfate and O_3 at a 1-day average. The fibrinolytic factor PAI-1 was significantly associated with increases in PM_{10}, $PM_{2.5}$, sulfate, and O_3 at 1- to 3-day averages, and nitrate at 2- to 3-day averages. By contrast, tPA, the other fibrinolytic factor, was not associated with any of these air pollutants. The coagulation factor fibrinogen was significantly associated with increases in PM_{10} at a 1-day average, and sulfate and O_3 at 1- to 3-day averages. There was no association between OC, EC, NO_2, CO, SO_2, and any of the blood markers (data not shown).

Changes in HRV Indices

Table 3 lists the associations between air pollution and HRV indices estimated by the single-pollutant mixed-effects models. On time-domain HRV indices, the single-pollutant mixed-effects models showed SDNN was significantly negatively associated with PM_{10}, $PM_{2.5}$, sulfate, nitrate, and O_3 at 1- to 3-day averages. r-MSSD was significantly negatively associated with sulfate, and nitrate at 1- to 3-day averages, and O_3 at 2- to 3-day averages. On frequency-domain HRV indices, the models showed LF was significantly negatively associated with PM_{10}, and $PM_{2.5}$ at 1- and 3-day averages, nitrate at a 1-day average, and sulfate and O_3 at 1- to 3-day averages. HF was significantly negatively associated with nitrate at a 1-day average, and sulfate and O_3 at 1- and 3-day averages. We found no associations between HRV indices and the other five air pollutants (i.e., OC, EC, SO_2, NO_2, and CO). The associations between air pollution and HRV indices remained after adjusting for the blood markers hs-CRP, fibrinogen, and PAI-1, separately, in the mixed-effects models.

Pollution Effects by Specific Components of Urban Air Pollution

Because single-pollutant mixed-effects models showed that sulfate and O_3 were more consistently associated with blood markers and HRV than the other eight air pollutants, we further performed two-pollutant mixed-effects models for these two pollutants at 1- to 3-day averages. Our two-pollutant models found that both sulfate and O_3 were significantly associated with increasing PAI-1 and decreasing SDNN and LF after adjusting to each other in the models. Moreover, sulfate was significantly associated with increasing 8-OHdG and fibrinogen, and decreasing r-MSSD and HF after adjusting for O_3. For brevity, we present only the modeling results with 3-day averaged pollution levels. As shown in Figure 1, 8-OHdG, PAI-1, and fibrinogen increased by 1.0, 5.1, and 1.3%, respectively, per IQR increase in 3-day averaged sulfate (4.3 $\mu g/m^3$). A 9.2% increase in PAI-1 was also found per IQR increase in 3-day averaged O_3 (16.0 ppb). SDNN, r-MSSD, LF, and HF were significantly decreased by 1.3 to 3.7% per IQR increase in 3-day averaged sulfate. SDNN and LF were significantly decreased by 4.0 to 4.9% per IQR increase in 3-day averaged O_3. The magnitude and pattern of pollution effects on health outcomes using 1-, 2-, or 3-day

TABLE 2. PERCENTAGE CHANGES IN INFLAMMATORY, OXIDATIVE STRESS, FIBRINOLYTIC, AND COAGULATION FACTORS FOR AN INTERQUARTILE RANGE CHANGE OF AIR POLLUTION LEVELS IN SINGLE-POLLUTANT MIXED-EFFECTS MODELS

Averaging Time (*days*)	PM_{10}	$PM_{2.5}$	Nitrate	Sulfate	O_3
hs-CRP					
1	135.8*	90.2	−2.1	80.0*	74.3
	(1.8 to 269.7)	(−10.2 to 190.1)	(−21.9 to 17.8)	(9.8 to 150.2)	(−9.0 to 157.7)
2	108.2	99.1	−11.6	87.1*	120.1*
	(−10.9 to 227.3)	(−26.1 to 224.3)	(−58.6 to 35.5)	(14.9 to 159.4)	(9.9 to 230.3)
3	109.6*	100.4	−18.7	71.1*	110.0*
	(2.5 to 216.7)	(−2.9 to 203.7)	(−69.9 to 32.5)	(13.0 to 129.2)	(2.7 to 217.3)
8-OHdG					
1	−9.2	−5.0	9.0*	1.0*	2.2*
	(−21.5 to 3.2)	(−14.3 to 4.4)	(4.0 to 14.1)	(0.3 to 1.3)	(0.9 to 3.5)
2	−6.1	−5.5	15.1*	−0.4	−6.9
	(−17.0 to 4.8)	(−15.6 to 4.6)	(5.9 to 24.3)	(−5.4 to 4.7)	(−15.7 to 1.9)
3	−5.6	−5.6	15.0*	−0.3	−4.2
	(−13.8 to 2.6)	(−13.8 to 2.6)	(4.9 to 25.0)	(−4.3 to 3.7)	(−12.6 to 4.3)
PAI-1					
1	30.0*	20.4*	4.0	12.0*	19.7*
	(12.4 to 47.7)	(17.3 to 33.5)	(−2.5 to 10.4)	(5.4 to 18.7)	(10.2 to 29.1)
2	19.1*	16.2*	11.6*	13.3*	30.9*
	(3.6 to 34.7)	(1.9 to 30.5)	(0.1 to 23.1)	(6.6 to 19.9)	(19.1 to 42.6)
3	21.2*	20.0*	16.9*	11.2*	33.0*
	(9.7 to 32.8)	(18.5 to 31.5)	(4.3 to 29.4)	(5.7 to 16.6)	(21.8 to 44.3)
tPA					
1	16.0	12.0	2.0	2.0	5.5
	(−4.1 to 36.2)	(−2.4 to 26.3)	(−6.2 to 10.3)	(−4.6 to 8.7)	(−4.4 to 15.4)
2	10.4	12.0	12.9	3.8	8.8
	(−6.3 to 27.2)	(−2.9 to 26.9)	(−1.6 to 27.5)	(−2.8 to 10.3)	(−3.8 to 21.6)
3	8.8	12.0	10.0	3.0	9.4
	(−2.8 to 20.5)	(−2.7 to 26.6)	(−5.8 to 25.8)	(−2.3 to 8.2)	(−2.8 to 21.5)
Fibrinogen					
1	5.3*	2.6	1.6	2.9*	4.8*
	(1.5 to 15.2)	(−2.7 to 7.8)	(−1.3 to 4.5)	(0.2 to 5.5)	(1.1 to 8.5)
2	1.5	1.5	1.3	2.8*	8.8*
	(−4.4 to 7.5)	(−4.1 to 7.1)	(−3.9 to 6.5)	(0.1 to 5.5)	(4.1 to 13.6)
3	3.3	3.6	1.0	2.2*	6.9*
	(−1.1 to 7.7)	(−0.8 to 8.1)	(−4.6 to 6.6)	(0.4 to 4.7)	(2.4 to 11.4)

Definition of abbreviations: 8-OHdG = 8-hydroxy-2′-deoxyguanosine; hs-CRP = high-sensitivity C-reactive protein; PAI-1 = plasminogen activator fibrinogen inhibitor-1; tPA = tissue-type plasminogen activator.

The values are presented as percentage changes and 95% confidence intervals for interquartile range changes after adjusting for sex, age, body mass index, weekday, temperature of the day before, and relative humidity in the models. The units for particulate air pollution and O_3 are $\mu g/m^3$ and ppb, respectively.

* $p < 0.05$.

averaged concentrations in two-pollutant models of our study were similar.

Significant correlations ($p < 0.001$) were observed between blood markers and HRV indices. The Pearson correlation analysis showed that PAI-1 was moderately correlated with SDNN ($r = -0.3$) and r-MSSD ($r = -0.4$); fibrinogen was moderately correlated with SDNN ($r = -0.6$), r-MSSD ($r = -0.4$), LF ($r = -0.5$), and HF ($r = -0.4$); and hs-CRP was moderately correlated with SDNN ($r = -0.3$).

DISCUSSION

This study provides evidence that urban air pollution is associated with systemic inflammation/oxidative stress, impairment of the fibrinolytic system, activation of blood coagulation, and alterations in autonomic nervous system in young, healthy humans. These cardiovascular effects occurred simultaneously after 1 day of exposure to urban air pollution in Taipei. Our study results support the viewpoint of the AHA's expert panel on biological mechanisms of air pollution effects on cardiovascular events. Gaseous and particulate air pollutants together with their soluble components may enter the lung and activate pulmonary neural reflexes and local inflammation to alter the autonomic nervous system, induce systemic inflammation/oxidative stress, and increase blood coagulability (1). Our findings are compatible with responses to air pollution observed in several previous studies. For instance, hs-CRP was induced by PM_{10} in an *in vitro* study (32). One recent panel study reported that increased levels of hs-CRP was associated with PM_{10} in patients with coronary heart disease (18). Fibrinogen in human studies was reported to be increased by PM_{10} (19, 20) and O_3 (4). The increases in 8-OHdG were reported to be induced by $PM_{2.5}$ in an *in vivo* study (29). The increases in fibrinogen and PAI-1 were associated with increases in O_3 (4) and urban air pollution (26). The decreases in HRV indices responded to increases in $PM_{2.5}$ (4–6, 8–10) and O_3 (4) levels in human studies.

There is growing evidence that increases in fibrinogen (33), hs-CRP (34), and PAI-1 (35) concentrations are associated with increases in risk of cardiovascular diseases. Recent epidemiological evidence links impaired endothelium-dependent vascular reactivity with cardiovascular diseases (36). It has been reported that high levels of hs-CRP and fibrinogen are associated with dysfunctional endothelium in cardiac patients (37, 38). Increased

TABLE 3. PERCENTAGE CHANGES IN HEART RATE VARIABILITY INDICES FOR AN INTERQUARTILE RANGE CHANGE OF AIR POLLUTION LEVELS IN SINGLE-POLLUTANT MIXED-EFFECTS MODELS

Averaging Time (*days*)	PM_{10}	$PM_{2.5}$	Nitrate	Sulfate	O_3
SDNN					
	−4.9*	−4.0*	−1.5*	−3.1*	−3.9*
1	(−7.8 to −2.1)	(−6.1 to −1.9)	(−2.6 to −0.3)	(−4.1 to −2.1)	(−5.4 to −2.4)
	−4.0*	−2.5	−2.6*	−4.1*	−6.6*
2	(−6.6 to −1.4)	(−4.6 to −0.4)	(−4.7 to −0.5)	(−5.2 to −3.1)	(−8.5 to −4.7)
	−4.1*	−3.0*	−3.0*	−2.0*	−8.3*
3	(−6.1 to −2.2)	(−5.0 to −1.1)	(−5.3 to −0.7)	(−2.9 to −1.2)	(−10.1 to −6.5)
r-MSSD					
	−4.8	−3.0	−5.5*	−5.0*	−3.6
1	(−12.3 to 2.7)	(−8.7 to 2.7)	(−8.7 to −2.2)	(−8.0 to −2.0)	(−7.7 to 0.6)
	−2.2	−2.0	−7.1*	−6.0*	−7.0*
2	(−9.0 to 4.7)	(−8.4 to 4.4)	(−14.0 to −0.2)	(−8.9 to −2.9)	(−12.3 to −1.6)
	−4.0	−3.6	−8.1*	−5.7*	−8.5*
3	(−9.0 to 0.9)	(−8.8 to 1.6)	(−14.5 to −1.8)	(−8.2 to −3.2)	(−13.6 to −3.3)
LF					
	−6.1*	−3.1*	−1.0*	−3.4*	−4.7*
1	(−10.1 to −2.1)	(−6.1 to −0.1)	(−1.6 to −0.5)	(−4.9 to −1.8)	(−6.8 to −2.5)
	−3.0	−3.2	−2.0	−3.0*	−6.0*
2	(−7.2 to 1.2)	(−4.6 to 0.1)	(−5.6 to 1.6)	(−4.5 to −1.5)	(−8.8 to −3.3)
	−4.3*	−3.4*	−2.0	−3.0*	−5.6*
3	(−7.0 to −1.6)	(−6.1 to −0.6)	(−5.2 to 1.2)	(−4.3 to −1.7)	(−8.2 to −3.0)
HF					
	−5.5	−3.7	−2.0	−3.5*	−3.6*
1	(−13.0 to 2.1)	(−9.4 to 2.1)	(−5.3 to 14)	(−6.5 to −0.4)	(−7.8 to −1.5)
	−2.7	−2.1	−4.9	−3.9*	−6.4*
2	(−9.5 to 4.1)	(−8.4 to 4.3)	(−10.9 to 0.9)	(−7.0 to −0.8)	(−11.7 to −1.0)
	−2.0	−4.0	−6.9*	−3.0*	−6.6*
3	(−7.2 to 3.2)	(−9.3 to 1.2)	(−13.4 to −0.3)	(−5.5 to −0.5)	(−11.8 to −1.4)

Definition of abbreviations: HF = high frequency; LF = low frequency; r-MSSD = square root of the mean of the sum of the squares of differences between adjacent NN intervals; SDNN = standard deviation of NN intervals.

The values are presented as percentage changes and 95% confidence interval for interquartile range changes after adjusting for sex, age, body mass index, weekday, temperature of the day before, and relative humidity in the models. The units for particulate air pollution and O_3 are $\mu g/m^3$ and ppb, respectively.

* $p < 0.05$.

hs-CRP concentration has been reported to induce PAI-1 expression and activity in human aortic endothelial cells (39), whereas increased PAI-1 expression was demonstrated in the media layer of the arterial segment with atherosclerotic changes (40) and considered as an acute reaction of the vascular endothelium, especially in atheromatous arteries (41). On the basis of these findings, we conclude that close relations exist between cardiovascular risk increase and impairment in endothelial function and vascular activity related to air pollution. Likewise, the increase of the likelihood of sudden cardiac death is related to

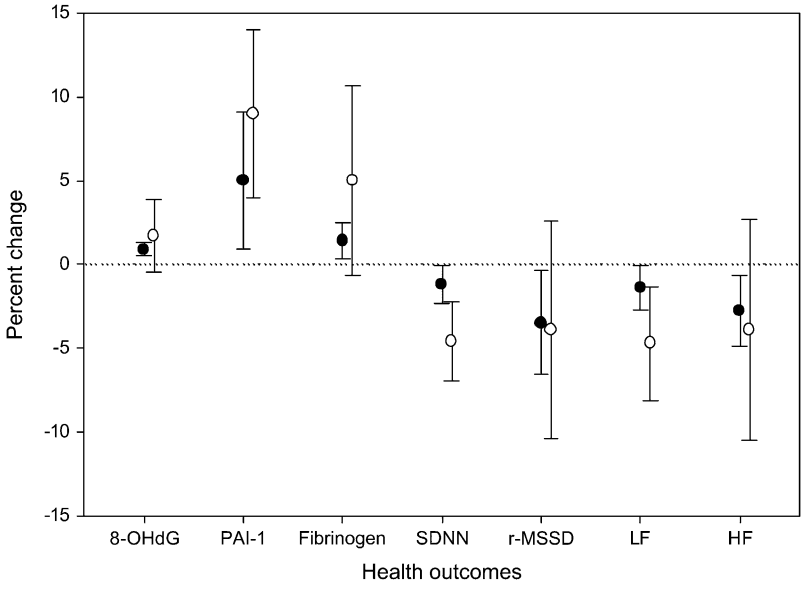

Figure 1. Percentage changes in blood markers and heart rate variability indices for interquartile range changes in 3-day averaged sulfate (*solid circles*) and ozone (*open circles*) levels among all participants estimated by two-pollutant mixed-effects models with sulfate and ozone adjusting for sex, age, body mass index, weekday, temperature of the day before, and relative humidity. *Error bars* indicate 95% confidence intervals. 8-OHdG = 8-hydroxy-2′-deoxyguanosine; HF = high frequency; LF = low frequency; PAI-1 = plasminogen activator fibrinogen inhibitor-1; r-MSSD = square root of the mean of the sum of the squares of differences between adjacent NN intervals; SDNN = standard deviation of NN intervals.

reduced HRV (42), which serves as a strong predictor of cardiac mortality after myocardial infarction (43). Because the cardiac autonomic alteration of both time-domain and frequency-domain HRV indices are observed in our study without major modification by blood markers, we believe that the risk of cardiovascular diseases might also be increased by decreases in autonomic nervous system control related to air pollution or the withdrawal of vagal activity. In a recent *in vivo* study, mice prone to developing atherosclerosis developed significant vascular inflammation and more lipid plaques in their arteries after a 6 months' exposure to concentrated $PM_{2.5}$. Such findings provide compelling evidence that long-term exposure to low concentrations of $PM_{2.5}$ can cause heart disease (44). By extrapolating our findings in healthy young people to patients with coronary heart disease, hypertension, or diabetes mellitus, we expect to see some adverse cardiovascular effects on these susceptible populations with short- and long-term exposure to urban air pollution.

Another interesting finding in our study is that sulfate emerged as the major particulate component contributing to $PM_{2.5}$-related cardiovascular endpoints. One source apportionment study reported that, in Taipei, vehicle emissions and secondary sulfate, the two most important sources of PM in urban environments, jointly account for 76.2% of mass concentrations of $PM_{2.5}$, with soil dusts, industrial emissions, and sea spraying accounting for the rest of $PM_{2.5}$ mass concentrations (45). Another study showed that sulfate was primarily formed by the oxidation of SO_2 from tailpipe emissions of diesel cars and trucks in Taipei (46), indicating that sulfate in $PM_{2.5}$ could be a good surrogate for traffic-related air pollution in our urban environment. Such findings also support the observation that sulfate in $PM_{2.5}$ is associated with cardiovascular mortality as reported in two previous population-based mortality studies (47, 48), with autonomic dysfunction as reported in one panel study (7), and with pulmonary inflammation as found in one controlled human study (49).

In addition, O_3 is another pollutant contributing to adverse cardiovascular effects in our study. Our findings are in agreement with ozone's association with cardiovascular mortality (50) and autonomic dysfunction as reported by others (4). It is known that O_3 is a major secondary pollutant formed by the photochemical oxidation of NO_2 and volatile organic compounds from automobile emissions. Because sulfate and O_3 are two typical secondary pollutants and represent similar groups or sources of pollutants in most urban air pollution, our findings imply that risk-based air pollution control policies should focus not only on emissions of primary pollutants but also on secondary aerosol precursors to maximize the benefits of health risk reduction.

Our study design also has its limitations. First, we used general environmental data to represent participants' exposures rather than personal monitoring data. Exposure misclassification or measurement error may have resulted in the lack of associations with OC, EC, and NO_2, which are considered to represent traffic sources. The reported slopes only indicate the association between air pollution and the biological outcome rather than the degree of change in the outcome due to direct exposure to the pollutant. Second, the blood samples and ECG monitoring for each study participant were collected at the same time in each measurement. Second, most of our markers for inflammation and blood coagulation are indicators of overall systemic response from blood rather than specific local responses, such as pulmonary inflammation from cells and fluid in bronchoalveolar lavage (15). Third, the outcome variables of ECG monitoring and blood samples in each study participant were not collected continuously over several hours within 1 day. Therefore, we cannot investigate the relation among the time sequence of inflammation, oxidative stress, blood coagulation, and autonomic dysfunction when people are exposed to urban air pollution. Last, a limited number of within-individual measurements (i.e., three times per participant) may not be sufficient to fully control the between-individual differences in health outcomes in our mixed-effects models.

Regardless of these limitations, our data still generally support the hypothesis that urban air pollution is associated with inflammation, oxidative stress, blood coagulation, and autonomic dysfunction simultaneously in humans. Further studies with more detailed measurements of cardiovascular endpoints over time are still needed to elucidate the time sequence of pollution effects on cardiovascular endpoints in humans.

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