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Association between nitrogen dioxide and heart rate variability in a susceptible population

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Background **Panel studies have shown a consistent association between changes in the cardiac autonomic nervous system with particulate matters (PM) but less with gaseous pollutants. This study examined the linkage between nitrogen dioxide (NO_2) and heart rate variability (HRV) in a susceptible population.**

Methods **We recruited a panel of 83 patients from the National Taiwan University Hospital Cardiology Clinic to measure their 24-h HRV by ambulatory electrocardiography. Thirty-nine patients had coronary heart disease (CHD) and another 44 patients had more than one major CHD risk factor. Ambient concentrations of NO_2, sulphur dioxide (SO_2), carbon monoxide (CO), ozone, and PM less than 10 μm in diameter (PM_{10}) at each participant's close-by monitoring station were used to represent study participants' exposures. We used linear mixed-effects models to analyse the association between individual air pollutants and $\log_{10}$-transformed HRV, with key personal and environmental attributes and co-pollutants being adjusted.**

Results **We found that an increase in 10 ppb NO_2 at 4-h to 8-h moving averages was associated with 1.5–2.4% decreases in the standard deviation of all normal-to-normal intervals (SDNN) in our participants. For every 10 ppb NO_2 at 5 and 7-h moving averages, our participants' low frequency was decreased by 2.2 and 2.5%, respectively. In contrast, HRV was not associated with PM_{10}, CO, SO_2, or O_3.**

Conclusion **Increasing NO_2 exposure was found to be associated with decreasing SDNN and low frequency in susceptible populations.** ***Eur J Cardiovasc Prev Rehabil*** **12:580–586 © 2005 The European Society of Cardiology**

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Introduction

The linkage between exposure to particulate air pollution and increased cardiovascular symptoms and mortality has been shown in epidemiological studies [1–3]. Several panel studies on particulate matter (PM) and heart rate variability (HRV) in susceptible human individuals have demonstrated that autonomic imbalance was a possible mechanism of PM-induced cardiovascular effects. Increased mass concentrations of PM less than 10 μm in diameter (PM_{10}) and PM less than 2.5 μm in diameter ($PM_{2.5}$) were associated with a decreased standard deviation of all normal-to-normal intervals (SDNN), square root of the mean of the sum of the squares of differences between adjacent normal-to-normal intervals (r-MSSD) in the elderly with coronary heart disease (CHD) or CHD risk factors, such as hypertension and diabetes mellitus [4–7]. Increased $PM_{2.5}$ mass concentrations were also related to the decrease in low frequency (LF; 0.04–0.15 Hz) and high frequency (HF; 0.15–0.40 Hz) in the elderly [8,9]. One recent study also found that increased PM less than 1 μm in diameter ($PM_{1.0}$) number concentrations were related to decreased SDNN, r-MSSD, LF and HF in the elderly and young subjects [10]. The time course of PM effects on HRV

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occurred between 1 and 8 h with most significant effects at 3–4 h after exposure.

By contrast, relatively few studies explored the effects of gaseous air pollutants on cardiovascular diseases. Previous studies showed that respiratory effects were associated with indoor [11] and outdoor [12] nitrogen dioxide (NO_2) exposure in children. In our studies, we also found that acute exposure to NO_2 was associated with increases in schoolchildren's illness absence and susceptible population's clinic visits for lower respiratory tract illness [13,14]. One epidemiological study recently reported that clinic visits for cardiovascular disease were associated with NO_2, carbon monoxide (CO), $PM_{2.5}$, organic carbon, elemental carbon, and oxygenated hydrocarbons [15]. Another epidemiological study examined the relationship between air pollution and the incidence of cardiac arrhythmia among 100 cardiac patients with implanted defibrillators. Particles, ozone, CO, sulphur dioxide (SO_2), and NO_2 were studied, but only NO_2 at lagged 1-day or 5-day moving average was associated with arrhythmia [16].

These findings led us to speculate that NO_2 may display similar effects on susceptible populations' HRV as did PM. Therefore, we designed this panel study to investigate whether exposure to NO_2 is associated with HRV of patients with cardiovascular disease or patients with risk factors for cardiovascular disease.

Methods

Participants

We recruited 83 patients aged 40–75 years from the cardiology section, Department of Internal Medicine, National Taiwan University Hospital, as our participants and conducted our panel study between 12 December 2001 and 21 February 2002. These participants included 39 patients with CHD and another 44 patients with more than one major CHD risk factor. Our 39 CHD patients included those who had history of angina pectoris or acute myocardial infarction, and had had cardiac catheterized and percutaneous transluminal coronary angioplasty during the past year, but excluded those who had been hospitalized or had thrombolytic therapy during the past 3 months before the study period. The 44 patients with CHD risk factors were those who had no angina pectoris symptoms and negative treadmill exercise tests, but had hypertension, hypercholesterolemia or diabetes mellitus. A total of 205 patients met our selection criteria, but only 83 agreed to participate in our study after we explained the monitoring protocols (response rate 40%). These 83 patients are all residents in Taipei metropolitan areas. The ethics committee of the National Taiwan University Hospital approved this study. An informed consent was obtained from each participant before the study started.

Continuous Holter monitoring and tape processing

We performed continuous ambulatory electrocardiographic (ECG) monitoring on each participant by using a three-channel ambulatory ECG recorder (PacerCorder, model 461A; Del Mar Medical Systems, LLC, Irvine, California, USA) with a sampling rate of 250 Hz (4 ms). We sent ECG tapes to National Taiwan University Hospital and used the Delmar 563 Holter analysis system (version 2.47; Del Mar Medical Systems) to do the analysis. The electrocardiographic wave complex (QRS) was automatically classified and manually verified as normal sinus rhythm, arterial or ventricular premature beats, or noise by comparison of the adjacent electrocardiographic wave complex morphological features. The normal-to-normal (N–N) intervals were deduced from the adjacent normal sinus beats. The N–N interval time series were then transferred to a personal computer and post-processed by a program written in Matlab language (version 5.2; MathWork Inc., Natick, Massachusetts, USA). The missing intervals of the raw N–N data were linearly interpolated and resampled at 4 Hz by the Ron Berger method [17]. Each 5-min segment of N–N intervals was taken for HRV analysis. The time-domain measurements of HRV were SDNN, and r-MSSD. The frequency-domain measurements of HRV included LF and HF, which were calculated using Welch's averaged periodogram of the N–N intervals [18,19]. In order to avoid sleep effects on HRV, we used 16-h daytime Holter measurements when the participants were awake between 0700 and 2300 h.

Environmental data

Concentrations of NO_2, CO, SO_2, ozone and PM_{10} measured by 12 fixed-site monitoring stations in Taipei metropolitan areas were used to represent our participants' exposures to air pollutants. These 12 air-monitoring stations, which were operated by Taiwan Environmental Protection Agency, were all located approximately 200–400 m away from main traffic roads in the school campus. The major sources of air pollution in Taipei, which is a metropolitan area without industries, are emissions from motorcycles, cars and buses. Each participant can be assigned to one fixed-site monitoring that is within 1 km of his or her residence. Hourly data on air pollution levels and temperature in each monitoring station during each participant's electrocardiogram monitoring period were obtained to represent personal exposure data.

Key personal attributes

Each participant's age, sex, body mass index, smoking status, and medical history were collected by a questionnaire. Each patient's current health status, including hypertension, diabetes mellitus and hypercholesterolemia was obtained from medical charts and examination. Professionally trained nurses performed blood pressure measurements for each patient with a

mercury sphygmomanometer. Participants with systolic blood pressure higher than 140 mmHg or diastolic blood pressure higher than 90 mmHg, or participants receiving antihypertension agents were considered to be hypertensive. Participants with fasting serum glucose levels of 6.99 mmol/l or greater in at least two different measurements or who had a history of taking the medicine were considered to be patients with diabetes. The US National Cholesterol Education Program – Adult Treatment Panel II guideline (NCEP-ATP II 1994) was adopted to define hypercholesterolemia to be low-density lipoprotein (LDL) cholesterol of 130 mg/dl or greater among CHD patients and LDL-cholesterol of 160 mg/dl among non-CHD patients.

Statistical analysis

We first plotted each participant's HRV indices against individual air pollutants to diagnose whether a consistent pattern existed between these two variables across all 83 participants, and whether some participants with extreme values biased such a pattern. We also used stepwise multiple regressions without air pollutants to determine key HRV-related personal covariates with a P value lower than 0.15 for further analyses. We then applied linear mixed-effects regression models, performed by S-PLUS 2000 general additive procedures (MathSoft Inc., Cambridge, Massachusetts, USA), to analyse the association between air pollutants and various HRV indices by adjusting key personal and environmental attributes. Such mixed-effects models had the advantage of adjusting for invariant variables in fixed-effects models and accounting for individual differences in random-effects models. We treated participants' sex, age, body mass index, health status (CHD versus non-CHD), smoking status (current versus never), medication use, and the hour of day as time invariant variables, whereas air pollutants, temperature and HRV were treated as time varying variables in our data analysis. Previous studies have shown that HRV reduction is associated with air pollution exposure in the preceding hours [4,10,20], we therefore evaluated the time course of pollution effects for 1–8 h after exposure. The time delay between clinical and environmental measurements was achieved by calculating moving averages of air pollution levels in 1–8 h before each 5-min HRV measurement. The $\log_{10}$-transformed HRV indices, which were 5-min SDNN, 5-min r-MSSD, 5-min LF and 5-min HF were then regressed on these moving averages. In the mixed-effects models, we treated participants' sex, age, body mass index, health status, smoking status, hour-of-day, 1–8 h moving averages of air pollutants, and ambient temperature as fixed effects and each participant as a random effect. Model selection was based on the criteria of minimizing Akaike's Information Criterion. Our mixed-effects models were applied separately to analyse all 83 participants as a whole and the diabetes mellitus participants as a subgroup.

Results

As shown in Table 1, there were 59 men and 24 women in our 83 study participants aged 40–75 years and the body mass index ranged from 19.5 to 31.6 kg/m^2. Among our study population, there were 39 patients with CHD, two patients with hypertension, 16 patients with hypercholesterolemia, one patient with hypertension and diabetes mellitus, 19 patients with hypertension and hypercholesterolemia, and six patients with hypertension, hypercholesterolemia and diabetes mellitus. Our participants' medication during the study period was 43 patients on beta-blockers, six patients on angiotensin-converting enzyme inhibitors, nine patients on calcium antagonists, and another 25 patients on other medication.

As shown in Table 2, the means (SD) of our participants' $\log_{10}$ HRV indices were 1.64 ms (0.31), 1.06 ms (0.37), 2.42 ms^2 (0.75), and 2.26 ms^2 (0.86) for SDNN, r-MSSD, LF, and HF, respectively. Table 2 also summarized the means (SD) of air pollution levels and meteorological conditions, which were 33.0 ppb (14.6), 1.1 ppm (0.9), 4.6 ppb (3.9), 21.9 ppb (15.4), 54.8 $\mu g/m^3$ (39.1), and 16.9°C (3.5) for NO_2, CO, SO_2, ozone, PM_{10}, and temperature, respectively, during our study period. In our air pollution data, NO_2 was strongly correlated with CO ($r = 0.7$) but moderately correlated with PM_{10} ($r = 0.4$), SO_2 ($r = 0.5$), and ozone ($r = -0.4$).

The diagrams of HRV versus air pollution indicated a consistently negative trend between all four HRV indices and four individual air pollutants, such as NO_2, PM_{10}, CO, and SO_2 across all 83 participants (not shown). A total of 160 single-pollutant models were constructed separately to estimate the effects of five air pollutants on HRV at 1-h to 8-h moving averages in this study. Table 3

Table 1 Summary of 83 participants' basic characteristics, health status, and medication in our panel study

Sex (no.)	
Female	24
Male	59
Age (years)	
Mean ± SD	60.6 ± 9.2
Range	40–75
Body mass index (kg/m^2)	
Mean ± SD	25.4 ± 25.4
Range	19.5–31.6
Smoking status (no.)	
Current smoker	37
Never smoker	46
Health status (no.)	
Coronary heart disease	39
Hypertension	2
Hypercholesterolemia	16
Hypertension and diabetes mellitus	1
Hypertension and hypercholesterolemia	19
Hypertension, hypercholesterolemia and diabetes mellitus	6
Medication (no.)	
Beta-blockers	43
Angiotensin-converting enzyme inhibitors	6
Calcium antagonists	9
Other medication	25

and 4 are estimated percentage changes of time-domain and frequency-domain HRV indices for air pollutant exposures at 1-h to 8-h moving averages by using single-pollutant mixed-effects models. The modeling results showed that NO_2 was associated with decreases of SDNN at 4-h to 8-h moving averages, and LF at 5-h and 7-h moving averages. The observed negative trend between NO_2 and r-MSSD and HF was, however, not significantly associated. Our single-pollutant models showed no significant association for the observed negative trends between time-domain and frequency-domain HRV indices and either PM_{10}, CO, or SO_2. The single-pollutant models further confirmed that no association existed between HRV indices and CO. For the seven participants with diabetes mellitus, we found that no air pollutants were significantly associated with HRV reduction, although all HRV indices consistently showed a negative correlation with NO_2 and CO.

We performed another 32 multi-pollutant models with PM_{10}, NO_2, and the interaction between PM_{10} and NO_2 in our models in order to estimate the partial effects of NO_2 on HRV adjusted for PM_{10} and other key personal and environmental attributes. Our multi-pollutant models found that only NO_2 had negative effects on SDNN and LF after adjusting PM_{10} and other key personal and environmental attributes. As shown in Fig. 1, SDNN decreased by 1.4–2.5% for every 10 ppb increase in 4-h to 8-h NO_2 moving averages. The LF decreased by 2.2% at 5-h moving averages and decreased by 2.4% at 7-h moving average per 10 ppb NO_2 exposure. In contrast, PM_{10} did not associate with any HRV indices in our multiple-pollutant models. Personal characteristics such as sex, age, body mass index, health status, medication use, and smoking status did not affect the relationship between NO_2 and time-domain and frequency-domain HRV indices. It should be noted that ambient temperature was negatively associated with these indices. We only examined the time course of air pollutant exposures up to 8-h moving averages because available HRV data were substantially reduced for moving averages greater than 8 h.

Table 2 Summary of heart rate variability, air pollution levels, and ambient temperature for 83 participants during the panel study period

Attributes	Mean ± SD (range)	No. of measurements
5-Min time-domain HRV (ms)		
Log_{10} SDNN	1.64 ± 0.31 (0.57–2.49)	1186
Log_{10} r-MSSD	1.06 ± 0.37 (0.32–2.04)	1186
5-Min frequency-domain HRV (ms^2)		
Log_{10} LF	2.42 ± 0.75 (0.04–4.99)	1186
Log_{10} HF	2.26 ± 0.86 (0.32–4.68)	1186
Air pollution levels (1-h moving average)		
PM_{10} ($\mu g/m^3$)	54.8 ± 39.1 (7.2–309.9)	1100
Ozone (ppb)	21.9 ± 15.4 (0.2–114.9)	949
NO_2 (ppb)	33.0 ± 14.6 (0.9–110.4)	1100
SO_2 (ppb)	4.6 ± 3.9 (0.1–26.3)	1110
CO (ppm)	1.1 ± 0.9 (0.1–7.7)	1119
Ambient temperature (1-h moving average, °C)	16.9 ± 3.5 (8.5–28.2)	1135

CO, Carbon monoxide; HF, high frequency; HRV, heart rate variability; LF, low frequency; NO_2, nitrogen dioxide; PM_{10}, particulate matter less than 10 μm in diameter; r-MSSD, square root of the mean of the sum of the squares of differences between adjacent normal-to-normal intervals; SDNN, standard deviation of all normal-to-normal intervals; SO_2, sulphur dioxide.

Discussion

This is the first study to demonstrate that environmental exposure to NO_2 is associated with reducing HRV in human subjects. The main effect of NO_2 on reducing HRV occurs at 4-h to 8-h moving averages for SDNN and 5-h and 7-h moving averages for LF. Our previous study reported that all HRV indices, namely, SDNN, r-MSSD,

Table 3 Percentage changes[a] of time-domain heart rate variability per unit concentrations of air pollutants lagged 1–8 h among 83 participants

	NO_2 (ppb)		PM_{10} ($\mu g/m^3$)		CO (ppm)		SO_2 (ppb)		Ozone (ppb)	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI	%	95% CI
SDNN										
1-h moving	−0.09	−0.20, 0.02	−0.01	−0.04, 0.02	−0.37	−2.14, 1.39	0.10	−0.28, 0.48	0.04	−0.07, 0.15
2-h moving	−0.10	−0.21, 0.03	−0.01	−0.05, 0.03	0.05	−1.98, 2.08	0.05	−0.35, 0.44	0.04	−0.08, 0.15
3-h moving	−0.10	−0.22, 0.02	−0.00	−0.04, 0.04	0.00	−2.20, 2.20	−0.06	−0.48, 0.37	0.05	−0.08, 0.18
4-h moving	−0.15*	−0.27, −0.01	−0.01	−0.05, 0.04	−0.69	−3.15 1.78	−0.14	−0.63, 0.35	0.09	−0.03, 0.21
5-h moving	−0.17*	−0.31, −0.03	−0.02	−0.07, 0.03	−1.34	−4.08, 1.41	−0.29	−0.82, 0.24	0.07	−0.06, 0.22
6-h moving	−0.17*	−0.32, −0.02	−0.03	−0.08, 0.02	−1.75	−4.81, 1.30	−0.25	−0.82, 0.32	0.05	−0.09, 0.20
7-h moving	−0.24*	−0.40, −0.08	−0.04	−0.11, 0.02	−2.23	−5.56, 1.12	−0.44	−1.07, 0.20	0.03	−0.11, 0.18
8-h moving	−0.23*	−0.40, −0.06	−0.04	−0.10, 0.02	−1.98	−5.60, 1.64	−0.47	−1.16, 0.22	0.01	−0.15, 0.16
r-MSSD										
1-h moving	−0.12	−0.30, 0.06	0.00	−0.05, 0.06	−0.43	−3.37, 2.50	0.10	−0.53, 0.74	0.08	−0.10, 0.27
2-h moving	−0.11	−0.30, 0.09	0.00	−0.07, 0.08	0.57	−2.85, 3.99	−0.07	−0.75, 0.61	0.06	−0.14, 0.26
3-h moving	−0.10	−0.28, 0.07	0.00	−0.06, 0.07	1.14	−2.68, 4.97	−0.10	−0.84, 0.64	0.10	−0.12, 0.32
4-h moving	−0.15	−0.35, 0.05	−0.00	−0.09, 0.09	0.12	−4.03, 4.27	−0.23	−1.04, 0.59	0.10	−0.10, 0.30
5-h moving	−0.15	−0.37, 0.06	−0.00	−0.08, 0.08	−0.61	−5.23, 2.00	−0.36	−1.25, 0.54	0.08	−0.13, 0.30
6-h moving	−0.13	−0.36, 0.11	−0.03	−0.12, 0.06	−0.87	−6.01, 4.27	−0.19	−1.16, 0.79	0.03	−0.20, 0.26
7-h moving	−0.20	−0.46, 0.06	−0.05	−0.15, 0.05	−1.06	−6.68, 4.55	−0.40	−1.50, 0.69	−0.06	−0.31, 0.18
8-h moving	−0.15	−0.44, 0.13	−0.06	−0.16, 0.04	0.08	−6.04, 6.20	−0.29	−1.50, 0.92	−0.12	−0.37, 0.13

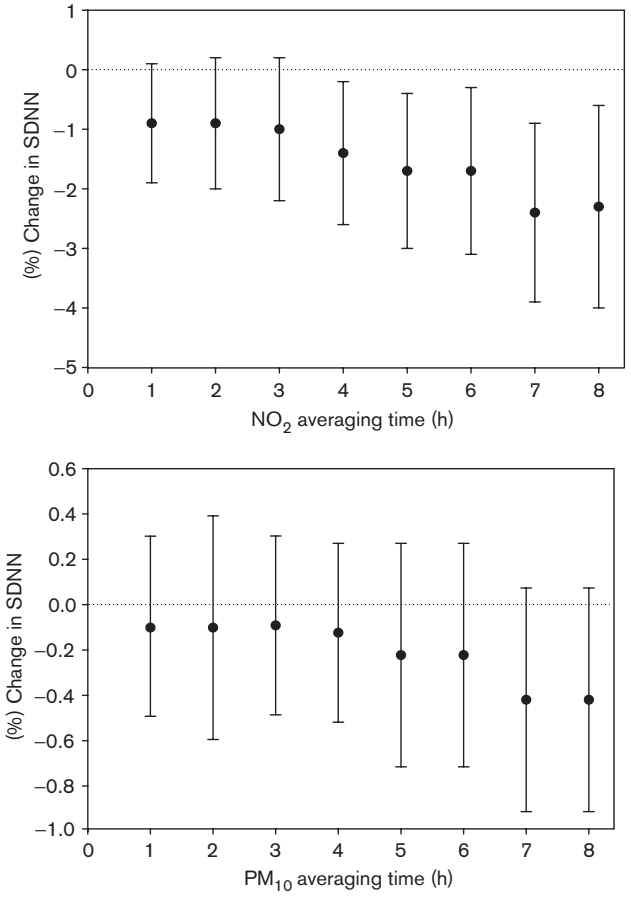
[a]The model was adjusted for sex, age, body mass index, health status, smoking status, medication use, indicator variables for hour of day, and ambient temperature. CI, confidence interval; CO, carbon monoxide; NO_2, nitrogen dioxide; PM_{10}, particulate matter less than 10 μm in diameter; r-MSSD, square root of the mean of the sum of the squares of differences between adjacent normal-to-normal intervals; SDNN, standard deviation of all normal-to-normal intervals; SO_2, sulphur dioxide. *$P<0.05$.

Table 4 Percentage changes[a] of frequency-domain heart rate variability per unit concentrations of air pollutants lagged 1–8 h among 83 participants

	NO_2 (ppb)		PM_{10} (μg/m^3)		CO (ppm)		SO_2 (ppb)		Ozone (ppb)	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI	%	95% CI
LF										
1-h moving	−0.07	−0.23, 0.09	0.00	−0.03, 0.03	1.14	−1.71, 3.97	0.00	−0.57, 0.57	0.11	−0.05, 0.27
2-h moving	−0.15	−0.33, 0.02	0.00	−0.03, 0.03	0.91	−2.31, 4.12	−0.31	−0.93, 0.31	0.16	−0.02, 0.34
3-h moving	−0.13	−0.30, 0.05	0.00	−0.03, 0.04	0.95	−2.60, 4.50	−0.50	−1.16, 0.16	0.19	−0.01, 0.39
4-h moving	−0.18	−0.37, 0.01	−0.00	−0.04, 0.04	−0.20	−4.10, 3.70	−0.56	−1.29, 0.18	0.10	−0.09, 0.29
5-h moving	−0.22*	−0.41, −0.04	0.00	−0.05, 0.05	−1.16	−5.49, 3.17	−0.75	−1.56, 0.07	0.08	−0.11, 0.28
6-h moving	−0.20	−0.41, 0.02	0.00	−0.03, 0.03	−1.14	−5.96, 3.69	−0.49	−1.37, 0.39	−0.01	−0.22, 0.20
7-h moving	−0.25*	−0.50, −0.01	−0.01	−0.06, 0.04	−0.81	−6.06, 4.43	−0.53	−1.51, 0.44	−0.08	−0.30, 0.14
8-h moving	−0.22	−0.48, 0.04	0.00	−0.06, 0.06	−0.20	−5.91, 5.50	−0.70	−1.77, 0.38	−0.10	−0.33, 0.13
HF										
1-h moving	−0.14	−0.32, 0.05	−0.02	−0.09, 0.05	0.79	−2.38, 3.96	0.07	−0.58, 0.72	0.14	−0.05, 0.34
2-h moving	−0.11	−0.31, 0.08	−0.02	−0.09, 0.05	1.54	−2.10, 5.17	−0.19	−0.88, 0.50	0.09	−0.13, 0.31
3-h moving	−0.05	−0.26, 0.17	−0.01	−0.08, 0.06	2.41	−1.71, 6.52	−0.24	−1.01, 0.53	0.12	−0.11, 0.35
4-h moving	−0.08	−0.29, 0.13	−0.02	−0.10, 0.06	1.70	−2.80, 6.20	−0.37	−1.23, 0.49	0.07	−0.14, 0.28
5-h moving	−0.12	−0.35, 0.11	−0.01	−0.09, 0.07	1.12	−3.84, 6.08	−0.59	−1.53, 0.35	0.04	−0.20, 0.28
6-h moving	−0.07	−0.32, 0.18	0.01	−0.09, 0.11	1.10	−4.40, 6.60	−0.39	−1.42, 0.63	−0.04	−0.28, 0.20
7-h moving	−0.09	−0.37, 0.18	−0.01	−0.12, 0.10	1.43	−4.56, 7.43	−0.44	−1.60, 0.71	−0.10	−0.36, 0.15
8-h moving	−0.04	−0.33, 0.25	−0.01	−0.12, 0.11	2.61	−3.98, 9.21	−0.45	−1.73, 0.81	−0.13	−0.40, 0.14

[a]The model was adjusted for sex, age, body mass index, health status, smoking status, medication use, indicator variables for hour of day, and ambient temperature. CI, confidence interval; CO, carbon monoxide; HF, high frequency; LF, low frequency; NO_2, nitrogen dioxide; PM_{10}, particulate matter less than 10 μm in diameter; SO_2, sulphur dioxide. *$P<0.05$.

Fig. 1



Percentage changes in 5-min standard deviation of all normal-to-normal intervals (SDNN) per 10 ppb nitrogen dioxide (NO_2) and 10 μg/m^3 particulate matter less than 10 μm in diameter (PM_{10}) estimated by multi-pollutant mixed-effects models.

LF, and HF, were negatively associated with submicrometer particle exposures in susceptible populations [10]. The comparisons between these two studies showed that NO_2 induced a smaller extent of HRV changes than submicrometer particles. Compared with previous studies on PM, our studies showed that NO_2 needed longer hours to induce SDNN reduction than PM. The time courses of reducing SDNN were 1-h to 4-h moving averages of PM exposures in previous studies [4,10,20]. In contrast, NO_2 induced an SDNN decrease only after 4-h moving averages in this study.

Findings of epidemiological, toxicological and controlled human exposure studies on NO_2 show that pulmonary inflammation is a possible mechanism to explain the observed associations between NO_2 exposure and autonomic imbalance in susceptible populations. Office workers' fibrinogen concentrations were associated with ambient NO_2 concentrations in London, UK [21]. Ambient NO_2 concentrations were also associated with an increase in platelet counts and fibrinogen among US adult populations [22]. An in-vitro study showed that the exposure of human bronchial epithelial cells to 0.4–0.8 ppm NO_2 for 6 h was associated with the synthesis of proinflammatory cytokines such as granulocyte–macrophage colony-stimulating factor, IL-8, and TNF-α [23]. One controlled human exposure study also showed that participants were detected with neutrophilic inflammation in their airways at 6 h after exposure to 2 ppm NO_2 for 4 h [24]. These findings support the theory that NO_2-induced pulmonary inflammation is one possible mechanism responsible for HRV decreases in susceptible populations.

In contrast, we found no effects of PM_{10}, ozone$_3$, CO or SO_2 on decreasing HRV in this study even though some previous studies have reported that PM_{10} [4,5], ozone

[4,8], CO [25], and SO_2 [26] were separately associated with an HRV decrease in various populations. One possibility of this discovery was that it was caused by the different spatial representativeness of fixed-site air-monitoring stations for NO_2 and other air pollutants [27]. In that study, we correlated hourly concentrations of NO_2, PM_{10}, CO, SO_2, and ozone measured at one fixed-site air-monitoring station with those measured at six mobile monitoring stations surrounding the fixed-site air-monitoring station and found that NO_2 had the highest correlation coefficients ($r = 0.73$). It is likely that NO_2, CO, SO_2, and ozone measured at the air-monitoring station may not properly represent our participants' air pollution exposures. The true association between these four air pollutants and HRV, therefore, may be biased towards null in this study. Accordingly, our study cannot completely falsify these four air pollutants' effects on decreasing HRV as reported in previous studies.

The following study limitation should be considered in the interpretation and extrapolation of our findings. First, the actual exposure–response relationship between NO_2 exposure and HRV decrease may be overestimated in this study because we used environmental monitoring data to represent individual exposures rather than personal monitoring data. Our patients' personal NO_2 exposures were expected to be higher than the NO_2 concentrations measured in air-monitoring stations because their breathing zones were closer to the emission sources of NO_2, such as vehicles outdoors and gas stoves indoors, than the monitoring station's sampling inlets [28]. Accordingly, the true exposure–response relationship between NO_2 exposure and HRV decrease should be lower than the findings of this study. Second, there may be some unknown and unmeasured air pollutants either indoors or outdoors, such as fine particles and combustion gases emitted from vehicles and cooking stoves. It is known that fine particles and nitrogen oxides are usually formed and emitted from tail-pipes together because of high-temperature combustions through vehicles' internal engines [29]. Third, other indoor air pollutants can also confound our results because our participants were expected to have their activities in indoor environments where indoor air pollution was not fully characterized in this study. Fourth, we cannot exclude the confounding effects of respiration on the association between NO_2 and HRV because our participants' physical activities and breathing patterns were not measured in the study [30]. Fifth, we believe that insufficient sample size is a possible reason why no air pollutants were found to be significantly associated with HRV reduction in the diabetes mellitus subgroup because we had only 168 HRV measurements for the seven patients with diabetes as outcomes in our mixed-effects models. Sixth, the between-participant variation in HRV, which is typically substantially larger than the within-participant variation, may not be fully controlled in our mixed-effects models and may lead to risk underestimation even though we have adjusted for as many invariant factors as possible, which included sex, age, health status, medication use and smoking status. Finally, we cannot evaluate the confounding effect of our participants' long-term exposure on our findings because we did not consider their accumulated exposure until the time of our clinical assessments in this study.

A decrease in HRV has been shown to be a predictor of increased mortality after a myocardial infarction [31] and has been related to sudden arrhythmic death [32]. Different autonomic influences on cardiovascular function are reflected by different HRV indices. SDNN is a broad measure that reflects overall changes in autonomic tone. r-MSSD is correlated with HF, which has been used to estimate parasympathetic nervous systems and is linked to respiratory influences. LF is believed to represent mixed sympathetic and parasympathetic influences [18]. As a significant HRV decrease by NO_2 was seen in both SDNN and LF but not in r-MSSD or HF in this study, we hypothesized that the effects of NO_2 on HRV reduction may be related to both the sympathetic and parasympathetic nervous systems.

Despite these limitations, we believe our data generally support the fact that NO_2 can disturb autonomic function in susceptible human individuals. Therefore, the interaction between PM and NO_2 should be considered in the study design in future studies on air pollution effects on HRV. Further studies are still needed in order to elucidate true biological mechanisms and the actual dose–response relationship for NO_2 effects on HRV decrease in the human population.

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