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Measurement of inflammation and oxidative stress following drastic changes in air pollution during the Beijing Olympics: a panel study approach

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Abstract

Ambient air pollution has been linked to cardiovascular and respiratory morbidity and mortality in epidemiology studies. Frequently, oxidative and nitrosative stress are hypothesized to mediate these pollution effects, however precise mechanisms remain unclear. This paper describes the methodology for a major panel study to examine air pollution effects on these and other mechanistic pathways. The study took place during the drastic air pollution changes accompanying the 2008 Olympics in Beijing, China. After a general description of air pollution health effects, we provide a discussion of panel studies and describe the unique features of this study that make it likely to provide compelling results. This study should lead to a clearer and more precise definition of the role of oxidative and nitrosative stress, as well as other mechanisms, in determining acute morbidity and mortality from air pollution exposure.

Keywords

panel study; oxidative stress; exhaled breath condensate; 2008 Olympics

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Conflicts of interest
The authors declare no conflicts of interest.

Introduction to the problem of air pollution health effects

This paper describes the rationale and methods for determination of changes in oxidative/ nitrosative stress levels in humans who participated in a panel study of air pollution effects during the Beijing Olympics of 2008. This study is known as the Health Effects of Air pollution Reduction Trial (HEART). We present the rationale and design for the overall study as well as the specific methodology for the oxidative stress experiments. Results will be presented in future papers.

Ambient (outdoor) air pollution comprises a complex aerosol of particulate matter (PM) in a gaseous pollutant milieu that includes ozone, carbon monoxide, nitrogen oxides, and sulfur dioxide. Particles are commonly size fractionated as ultra-fine (<0.1 μm), $PM_{2.5}$ (<2.5 μm), coarse ($PM_{2.5}$–PM_{10}), and PM_{10} (<10 μm). Within and between size fractions, and also from location to location, PM content varies greatly in organic compound and metal speciation, largely dependent on whether the particle source is crustal, vegetative (pollen), or from combustion (often the smallest particles).

Substantial epidemiologic evidence documents that daily changes in air pollution levels, particularly but not exclusively its PM components, are associated with variations of one to a few percent in both mortality and morbidity from cardiovascular and respiratory causes.[1,2] Although these mortality and morbidity outcomes are also observed when making spatial comparisons/evaluations (i.e., comparing outcomes associated with chronic exposures between geographic regions/cities), they are especially prominent when making temporal comparisons (changes over time within the same population/ city). Many studies demonstrate significant variation in short-term (daily) rates of cardiopulmonary outcomes such as myocardial infarction and heart failure exacerbations.[2–4]

We have focused on these short-term variations in an effort to elucidate mechanisms by which air pollution acutely triggers biochemical and cellular events that underlie clinically observed outcomes. We have attempted to develop and refine mechanistically relevant correlates or biomarkers for such acute effects of pollutants.[5–7] One example of this is depicted in Figure 1, demonstrating an acute increase in the atypical biomarkers of right ventricular diastolic and pulmonary artery pressures in heart failure patients on the same day as an acute increase in ambient $PM_{2.5}$ near their residences. This was done in a unique panel of heart failure patients who had permanently implanted right ventricular pressure transducers.[5]

Mechanisms/biomarkers of concern for ambient air pollution

A number of interrelated pathways have been proposed to explain the epidemiologic phenomenon of air pollution induced acute mortality and morbidity, most of which have some corroborating, but not conclusive, supporting evidence. A diagram that summarizes the hypothesized effects of air pollution on various pathways is shown in Figure 2, along with relevant examples of biomarkers for each endpoint, as chosen for this study. Inhalation of particles is widely felt to induce inflammation both locally, in the respiratory tract, as well as probably systemically.[8] It is also felt to affect autonomic nervous system tone, altering sympathetic and parasympathetic output, possibly affecting the stability of atherosclerotic

plaques, and clearly decreasing heart rate variability (HRV).[9] Decreased HRV is a well-established risk for arrhythmia and other causes of cardiovascular mortality. Other potential mechanisms include acute changes in vascular or endothelial function[10] and the tendency of blood to coagulate, either due to platelet alterations or to changes of the soluble clotting system.[11] Biomarkers of oxidative stress are discussed in more detail later.

Panel studies

As mentioned earlier, one of the epidemiologic designs used to elucidate such key mechanistic pathways is the panel study, a smaller more intensively sampled variation of the cohort study. Panel studies have been used frequently to study air pollution health effects.[12,13] In this design a group of individual subjects is followed longitudinally forward in time, allowing serial (usually weekly or monthly but actually many times per day by telemetry in the above heart failure example), collection of health endpoint data, usually analyzed as a function of daily changes in ambient levels of pollution. Although some panel studies collect symptom or other self-report information, most collect biological specimen data from blood, breath, urine, or some other biological medium for laboratory analysis. Panels are typically smaller than cohort or case-control designs using tens, rather than hundreds or thousands of subjects. In air pollution studies investigators use typical ambient pollution as the independent variable, however this design has occasionally been modified to incorporate the more substantial pollution changes that accompany natural disasters.[14,15] To our knowledge, previous studies of natural experiments have never been accomplished in a "pre-during-post" (ABA) design where exposures and outcomes are measured at a baseline, then again following a change in pollution, and finally repeated after an expected return to baseline exposure conditions. In addition, this study is novel in its use of serial noninvasive measurement of exhaled breath biomarkers for assessment of pollution-associated changes in oxidative stress levels.

Exhaled breath markers of oxidative stress

As stated earlier, ambient air pollution is likely to induce oxidative stress as a mechanism leading to health effects.[16] Oxidative stress is felt to be a primary mechanistic link between environmental or pathologic stimuli and inflammation, although the inflammatory response can actually produce oxidative stress through release of cellular mediators. It may occur in tissues directly exposed to air pollution (the respiratory tract) as well as systemically,[17,13] presumably through inflammatory mechanisms or systemic transport of metals and other toxicants from the alveoli. Assessment of oxidative stress is typically done through measurement of biomarker molecules that directly reflect oxidative balance (NO, NO_2, NO_3), or molecules that are formed by the action of oxidants on lipids (e.g., malondialdehyde, 8-isoprostane) or nucleic acids (e.g., 8-hydoxydeoxyguanosine or 8-OHdG).

Numerous studies document that nitric oxide (NO) in exhaled breath (eNO) is increased among asthmatics, attributed to airway inflammation. This has been shown to fluctuate with the severity and treatment of their asthma.[18] However, no studies of this marker have been conducted in healthy subjects, using fluctuations in ambient pollution as the independent

variable. In addition to exhaled NO, a relatively well-established biomarker of asthma status, both nitrite (NO_2) and nitrate (NO_3) can be found in the respiratory tract. These are metabolic oxidation products of NO, produced primarily in the lung by inducible nitric oxide synthase (iNOS). As proximate and relatively stable metabolites of NO, they are excellent candidates to use as serial biomarkers of the level of pulmonary oxidative/nitrosative stress,[19,20] although concern has been raised about contamination from the oropharyngeal sources of nitrite.[21] Because they are not gaseous as is NO, and tend to be dissolved in the airway lining fluid, NO_2/NO_3 are most commonly measured in exhaled breath condensate (EBC) rather than in breath itself.[20,22] A decline in EBC pH has also been proposed as a marker of oxidative stress in the airways. Exhaled breath is saturated with water vapor and thought to reflect the airway lining fluid. EBC can be noninvasively collected by passing expired air through a cooling device. The EBC is condensed when passed through the cooling apparatus, and volatile and nonvolatile molecules can be assayed from the resultant fluid. Collection generally takes 5–15 min of tidal breathing for a yield of 1–3 mL of fluid.

This report documents our approach to the study of acute changes in biomarkers of pulmonary oxidative and nitrosative stress during a massive “natural” experiment that occurred in Beijing, China, during the 2008 Olympics. We sought to demonstrate changes in exhaled breath markers of pulmonary oxidative and nitrosative stress as a function of this regulatory experiment.

Beijing Olympics HEART study design

Beijing, China, is well known to have much higher air pollution levels than western developed cities, largely due to burgeoning, yet inadequately controlled, fossil fuel combustion by vehicles and stationary sources.

As one of its commitments to win the 2008 Summer Olympic and Paralympic Games, the Chinese government used its authoritarian powers to control air pollution sources in an effort to ensure that one of theworld’smost polluted cities would have ambient air quality during the Games comparable to that in typical Western cities. The control actions were implemented using a tiered series of actions. Tier 1 actions were implemented from July 20 to September 17 (starting 2 weeks before the Olympic opening ceremony and ending after the Paralympics) and included actions listed in Table 1. Their implementation is shown graphically in Figure 3.

These temporary air quality improvements provided an opportunity to address critical questions regarding acute biological mechanisms of cardiovascular effects related to ambient pollution over a uniquely broad concentration range.

We designed a panel study to examine the following hypotheses: (1) $PM_{2.5}$ and $PM_{2.5}$ constituents would drastically decline during the Olympic period, relative to the pre-Olympic period; (2) biomarkers of pulmonary and systemic inflammation and oxidative stress would change significantly during the Olympic air pollution reduction period, compared to the pre-Olympic period; (3) these biomarkers would likely return to pre-Olympic levels following relaxation of the air pollution controls in the post-Olympic period;

and (4) $PM_{2.5}$ and certain $PM_{2.5}$ constituents would each be associated with specific biomarkers across the whole study period. Table 2 lists all of the measured biomarkers.

This study was carried out at two collaborating institutions, the University of Medicine and Dentistry of New Jersey (UMDNJ) and the Peking University in Beijing. The study protocol was approved by both the Institutional Review Board of UMDNJ and the joint Ethics Committee of Peking University Health Science Center and the Peking University 1st Hospital in central Beijing. The panel was a homogenous group of nonsmoking Chinese hospital medical residents (students) with age between 25 and 35 years old, who are generally healthy and work and reside on or near the campus of the Beijing University 1st Hospital, close to central Beijing. We recruited 137 study participants through on-site advertising. We excluded individuals who are current smokers or have any of the following conditions: chronic respiratory, cardiovascular, liver, renal, hematological disease, diabetes mellitus, and other systemic diseases. As several participants withdrew after one or two visits due to schedule conflicts, a total of 128 nonsmoking healthy subjects were recruited to comprise the panel in this study.

We defined the study period from July 21 to August 29, 2008 as the “during-Olympic period,” with the period before this called “pre-Olympic period (June 2 to July 7)” and after called the “post-Olympic period (August 28 to October 31).” We selected this relatively narrow time window (~5 months) to limit potential seasonal confounding, but allow us to make health and biological measurements twice on each subject in each period for all 128 subjects. We used the central 28 days within each time period for health measurements, making two separate measurements in each period on each subject. Within each time period, each of the 128 subjects had a suite of health measurements made repeatedly on two separate days, separated by at least 2 weeks. Each subject was scheduled to have measurements made on the same day of the week within and across all the three periods. To minimize any potential impact from altered sleep patterns and activities, session days were scheduled during routine time/activity periods and not to follow a night shift or travel event. Moreover, the two repeated measures within each time period reduces the impact of potential variability of health endpoints within periods and individuals.

Exposure measurements

We conducted a comprehensive characterization of air pollution, covering and extending past the entire time window of the panel study, at a fixed location on the campus of the 1st Hospital. We started measurements 1week before the commencement of the subjects’ clinical measurements in the hospital. All the air samplers and monitors were collocated at a secured spot on the hospital campus. All the real-time (continuous) monitors were operated continuously throughout the entire measurement period (~6 months). The integrated measurements of PM_{10} and $PM_{2.5}$ mass concentrations as well as $PM_{2.5}$ constituents were made on a 24-h basis, everyday during each of the study period.

The real-time $PM_{2.5}$ mass concentration was monitored by Tapered Element Oscillating Microbalance (TEOM 1400a Ambient Particulate (PM-2.5)), at a flow rate of 16.7 L/min. Gaseous pollutants SO_2, NO_2, CO, and O_3 were monitored by EC9800 series ambient gas

analyzers (EcoTech, Australia).All real-time instruments were calibrated between 24:00 and 1:00 a.m. on daily basis. Temperature and relative humidity sensors were collocated with all air monitors.

A Quad-Channel ambient particulate sampler (TH-16A) was used in the field to collect a set of four Teflon and Quartz filters at size cut of PM_{10} and $PM_{2.5}$, every 24 h between 10 and 10 a.m. the next morning. The filters were used first to determine PM_{10} and $PM_{2.5}$ mass concentration gravimetrically, and were then analyzed for $PM_{2.5}$ composition. Elemental and organic carbon on quartz filters were analyzed using a Sunset OC/EC analyzer (Sunset Laboratory Inc., OR,USA) according to National Institute for Occupational Safety and Health guidelines (method 5040). A total of eight water-soluble anions and cations (Cl^-, NO_3^-, SO_4^{2-}, K^+, Ca^{2+}, Na^+, Mg^{2+}, NH_4^+) in one Teflon filter were determined by using ion chromatography (Dionex, DX2500). A total of 18 trace elements (Na, Mg, K, Ca, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Mo, Cd, Ba, Pb) on a Teflon filter were quantified by Inductively Coupled Plasma Mass Spectrometer (ICP-MS) using an Agilent 7500C ICP-MS instrument with a plasma forward power of 1350 W. Particle-phase polycyclic aromatic hydrocarbons (PAHs) collected on a quartz fiber filter were analyzed using GC/MS.

Clinical measurements

After recruitment and informed consent, each subject completed a medical history, physical examination, routine blood chemistries, spirometry, and ECG to rule out any medical conditions that would preclude participation. Each experimental session was about 60min in duration for each subject and occurred at the same time of day (early morning) on the same day of the week. Participants were asked not to use aspirin or NSAIDs for 2 weeks before testing to minimize possible interference with effects mediated by inflammation or inhibition of platelet function. Subjects could not have an active upper respiratory illness (either infection or allergy), and would be rescheduled if they had symptoms in the past 7 days. They could not have used anti-inflammatory medication for allergies or other respiratory conditions for 1 week prior to a session. On the day of each experimental session, the participants first underwent an electro-cardiogram (ECG) performed in the supine position for determination of HRV. Blood was drawn following ECG. Exhaled breath samples were collected following ECG measurement and phlebotomy. Exhaled breath condensate (EBC) from tidal breathing was collected from each subject in the seated position using a commercial breath-condensate collector (EcoScreen, Erich Jaeger, Germany). Approximately 2 mL of condensate were obtained per subject per visit for pH, nitrite and nitrate, and 8-isoprostane analysis. EBC pH was measured after de-aeration with argon using an electronic pH meter. EBC nitrite and nitrate were analyzed using a HPLC system (Waters Model 2695, USA) with a UV detector (Waters Model 2996, USA). Concentration of 8-ISP in EBC was measured by enzyme-linked immunosorbent assay (ELISA) based assay (Cayman Chemicals, USA). Exhaled nitric oxide (eNO) from functional residual capacity was collected through controlled expiration from each subject in the seated position into a NO-impermeable aluminum foil bag (Huayuan Gas Center, China). The eNO was analyzed by a NO_x chemiluminescence analyzer (Model 42C NO-NO2-NOX Analyzer, Thermo) within 3 h of collection.

Preliminary results and discussion

In general, the air pollution control measures implemented for the Beijing Olympics resulted in a reduction in PM and gaseous pollutant concentrations of 30–60% from the pre-Olympic period. Pulmonary and systemic inflammation, as well as oxidative stress, all demonstrated changes in the hypothesized directions (decreased inflammation and oxidative stress), from the pre-Olympic to the during-Olympic period. Detailed results for oxidative stress and other outcomes will be presented in subsequent papers.

This “real world” and natural experiment expands on previous acute studies on the adverse effects of air pollution by incorporating: a greater change in pollutant concentrations, reversibility of biological effects associated with pollution change, a larger suite of measured PM chemical constituents, and planned redundancy of outcome biomarkers for enhanced validation of results (e.g., multiple markers of platelet function, systemic inflammation, and oxidative stress). Our preliminary conclusions are that *in vivo* biomarkers of oxidative stress, especially pH, nitrite, and nitrate in exhaled breath condensate, are developing into key tools for the investigation of air pollution health effects.

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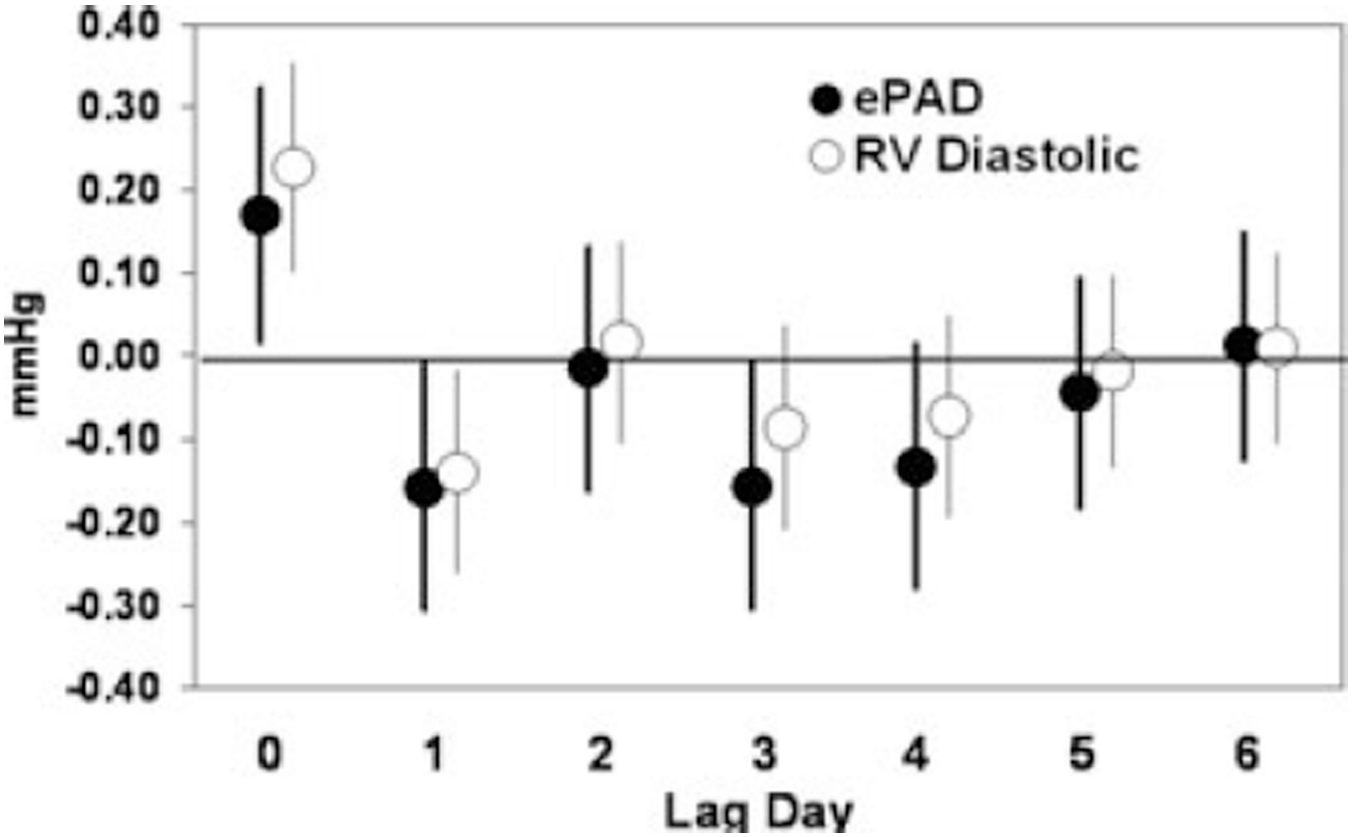

Figure 1.

Change and 95% confidence interval(s) in daily mean estimated pulmonary artery diastolic pressure (ePAD)and right ventricular diastolic (RVD) pressure (mmHg) associated with each 11.62 μg/m3 increase in mean daily PM2.5 concentration on lag days 0 to 6. A significant increase in pressures is seen on the same day as the pollution change.

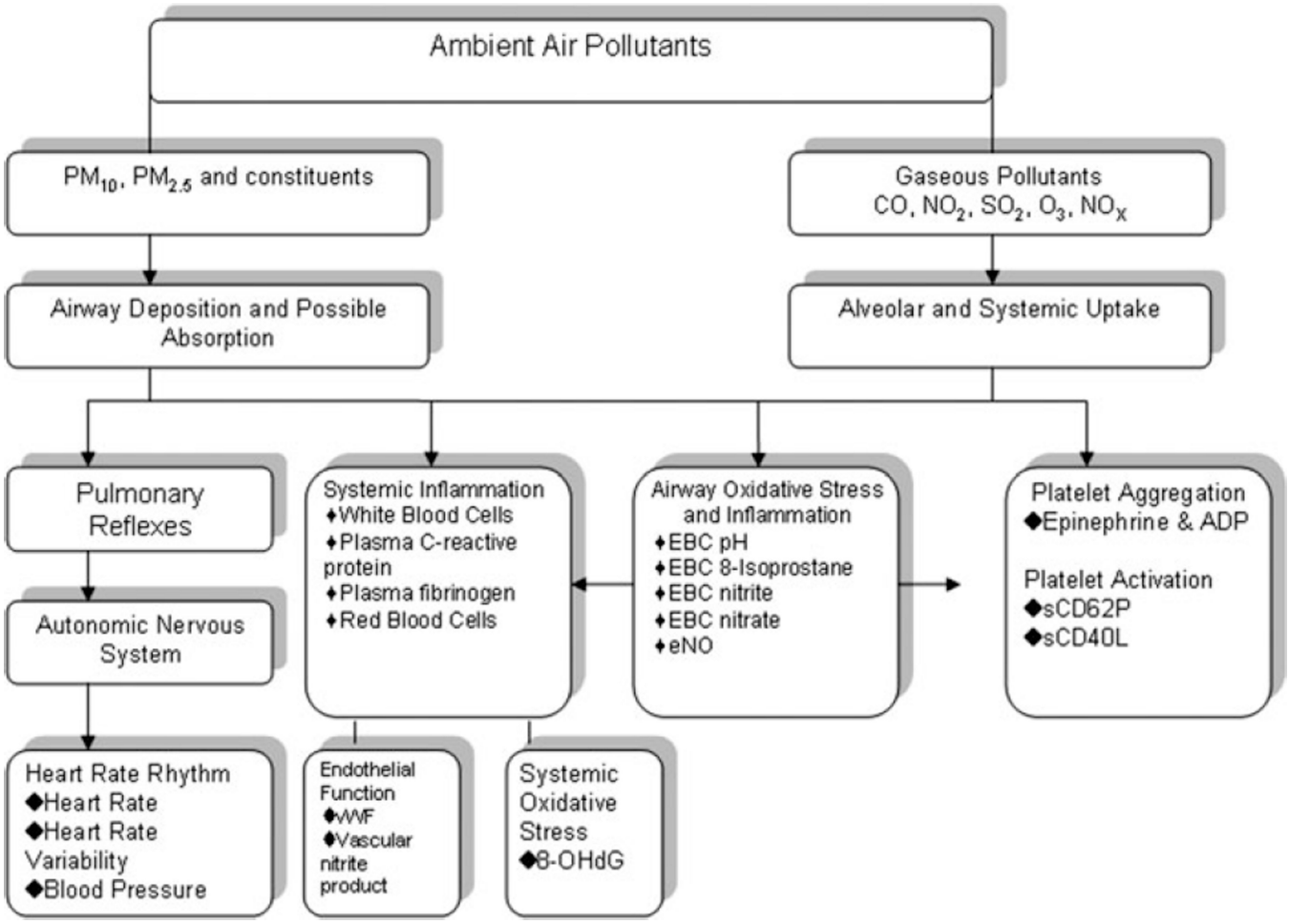

Figure 2.

Chart demonstrating hypothesized mode of action of air pollution particles with listing of relevant biomarkers used in the HEART study.

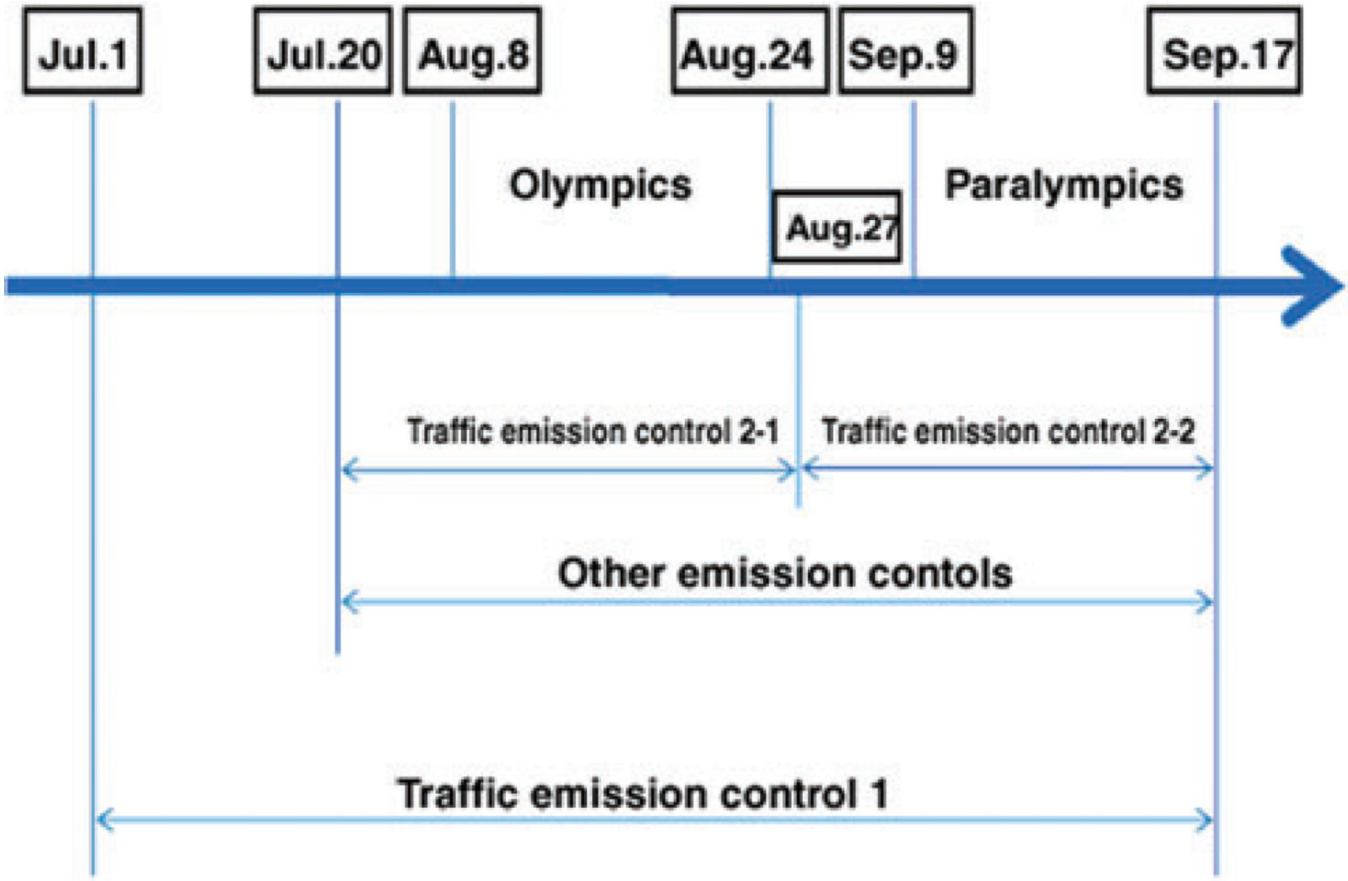

Figure 3.
Depiction of 2008 Beijing emission control implementation schema overlaid on sampling dates for the Olympics and for the HEART study.

Table 1

Emission controls during Beijing Olympics

- *Traffic emission control 1 (July 1st–September 17th)*: All vehicles that failed to meet the European No. I standards for exhaust emissions (including light-duty and heavy-duty trucks and inefficient personal vehicles) were banned from Beijing's roads
- *Traffic emission control 2 (July 20th–September 17th)*: Mandatory restrictions were implemented for personal vehicles, allowing them on roads only on alternate days depending on license plate numbers (odd-numbered vehicles on odd-numbered days and even-numbered vehicles on even-numbered days)
    - July 20th–August 27th: All vehicles followed the restrictions within (including) the sixth ring
    - August 28th–September 17th: Trucks could run outside (including) the fifth ring without restriction but not for all other vehicles
- *Other emission controls (July 20th–September 17th)*: In addition to traffic emission controls, other area and point sources in Beijing were placed under strict control during the Olympics period
    - Power plants in Beijing were required to reduce their emissions by 30% from their levels in June when they had already met the Chinese emission standard
    - Several heavily polluting factories were ordered to reduce their operating capacities or to completely shut down during the Games
    - All construction activities were placed on hold
    - Since it has been shown that Beijing's air-quality problems also have regional causes (Streets *et al.*, 2007;Wang *et al.*, 2008), emission controls on large industrial sources were also applied in surrounding provinces (e.g., Inner Mongolia, Shanxi, Hebei, Shandong) and in the city of Tianjin

Table 2

Biomarkers used in the HEART study

Physiological function/ pathway domain	Specimen type	Biomarker	Principle of measurement
Pulmonary inflammation and oxidative stress	Exhaled breath condensate	pH 8-Isoprostane Nitrite + nitrate	pH meter ELISA-based assay HPLC-UV
	Exhaled breath	Exhaled nitric oxide (eNO)	NO_x chemiluminescence analyzer
Autonomic tone	N/A	Heart rate variability (HRV)	Holter analysis systems
Endothelial function	Blood	Vascular NO production-blood nitrite	HPLC-UV
Endothelial-derived procoagulation	Blood	von Willebrand Factor	Immunoturbidimetry
Platelet function	Blood	Soluble CD62P sCD40L Platelet aggregation(ADR and ADP)	ELISA-based assay ELISA-based assay Photometric aggregometer
Systemic inflammation	Blood	White blood cell	Standard automated clinical methods
		Plasma C-reactive protein	Ratenephelometry
		Plasma fibrinogen	Clauss fibrinogen assay
Systemic oxidative stress	Urine	8-Hydroxy-2′-deoxyguanosine	HPLC-ECD