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Cardiorespiratory Biomarker Responses in Healthy Young Adults to Drastic Air Quality Changes Surrounding the 2008 Beijing Olympics

Junfeng Zhang, Tong Zhu, Howard Kipen, Guangfa Wang, Wei Huang, David Rich, Ping Zhu, Yuedan Wang, Shou-En Lu, Pamela Ohman-Strickland, Scott Diehl, Min Hu, Jian Tong, Jicheng Gong, and Duncan Thomas

Includes a Commentary by the Institute's Health Review Committee

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CONTENTS

ABOUT HEI

The Health Effects Institute is a nonprofit corporation chartered in 1980 as an independent research organization to provide high-quality, impartial, and relevant science on the effects of air pollution on health. To accomplish its mission, the institute

- Identifies the highest-priority areas for health effects research;
- Competitively funds and oversees research projects;
- Provides intensive independent review of HEI-supported studies and related research;
- Integrates HEI's research results with those of other institutions into broader evaluations; and
- Communicates the results of HEI's research and analyses to public and private decision makers.

HEI typically receives half of its core funds from the U.S. Environmental Protection Agency and half from the worldwide motor vehicle industry. Frequently, other public and private organizations in the United States and around the world also support major projects or research programs. HEI has funded more than 280 research projects in North America, Europe, Asia, and Latin America, the results of which have informed decisions regarding carbon monoxide, air toxics, nitrogen oxides, diesel exhaust, ozone, particulate matter, and other pollutants. These results have appeared in the peer-reviewed literature and in more than 200 comprehensive reports published by HEI.

HEI's independent Board of Directors consists of leaders in science and policy who are committed to fostering the public–private partnership that is central to the organization. The Health Research Committee solicits input from HEI sponsors and other stakeholders and works with scientific staff to develop a Five-Year Strategic Plan, select research projects for funding, and oversee their conduct. The Health Review Committee, which has no role in selecting or overseeing studies, works with staff to evaluate and interpret the results of funded studies and related research.

All project results and accompanying comments by the Health Review Committee are widely disseminated through HEI's Web site (*www.healtheffects.org*), printed reports, newsletters and other publications, annual conferences, and presentations to legislative bodies and public agencies.

ABOUT THIS REPORT

Research Report 174, *Cardiorespiratory Biomarker Responses in Healthy Young Adults to Drastic Air Quality Changes Surrounding the 2008 Beijing Olympics*, presents a research project funded by the Health Effects Institute and conducted by Dr. Junfeng (Jim) Zhang of the University of Medicine and Dentistry of New Jersey–School of Public Health, and the Environmental and Occupational Health Sciences Institute, Rutgers University, Piscataway, N.J., and his colleagues. This report contains three main sections.

The HEI Statement, prepared by staff at HEI, is a brief, nontechnical summary of the study and its findings; it also briefly describes the Health Review Committee's comments on the study.

The Investigators' Report, prepared by Zhang and colleagues, describes the scientific background, aims, methods, results, and conclusions of the study.

The Commentary is prepared by members of the Health Review Committee with the assistance of HEI staff; it places the study in a broader scientific context, points out its strengths and limitations, and discusses remaining uncertainties and implications of the study's findings for public health and future research.

This report has gone through HEI's rigorous review process. When an HEI-funded study is completed, the investigators submit a draft final report presenting the background and results of the study. This draft report is first examined by outside technical reviewers and a biostatistician. The report and the reviewers' comments are then evaluated by members of the Health Review Committee, an independent panel of distinguished scientists who have no involvement in selecting or overseeing HEI studies. During the review process, the investigators have an opportunity to exchange comments with the Review Committee and, as necessary, to revise their report. The Commentary reflects the information provided in the final version of the report.

PREFACE

HEI's Outcomes Research Program

The goal of most air quality regulations is to protect the public's health by implementing regulatory actions or providing economic incentives that help reduce the public's exposure to air pollutants. If this goal is met, air pollution should be reduced, and indicators of public health should improve or at least not deteriorate. Evaluating the extent to which air quality regulations succeed in protecting public health is part of a broader effort — variously termed *outcomes research*, *accountability research*, or *research on regulatory effectiveness* — designed to assess the performance of environmental regulatory policies in general. In recent decades, air quality in the United States and Western Europe has improved substantially, and this improvement is attributable to a number of factors, including increasingly stringent air quality regulations. However, the cost of the pollution-control technologies and mechanisms needed to implement and enforce these regulations is often high. It is therefore prudent to ask whether the regulations have in fact yielded demonstrable improvements in public health, which will provide useful feedback to inform future efforts.

Several U.S. government agencies have concluded that direct evidence about the extent to which air quality regulations have improved health (measured as a decrease in premature mortality and excess morbidity) is lacking. This finding is well documented by the National Research Council (NRC) in its report *Estimating the Public Health Benefits of Proposed Air Pollution Regulations* (NRC 2002), as well as by the California Air Resources Board, the U.S. Environmental Protection Agency (EPA), the U.S. Centers for Disease Control and Prevention (CDC), and other agencies.

In 2003, the Health Effects Institute published a monograph on outcomes research, Communication 11, *Assessing Health Impact of Air Quality Regulations: Concepts and Methods for Accountability Research* (HEI 2003). This monograph was written by the members of HEI's multidisciplinary Accountability Working Group after a 2001 workshop on the topic. Communication 11 set out a conceptual framework for outcomes research and identified the types of evidence required and the methods by which the evidence should be obtained. It has also guided the development of the HEI Health Outcomes Research program, which is discussed below.

Between 2002 and 2004, HEI issued four requests for applications (RFAs), under which eight studies were funded (see Table). The study by Junfeng (Jim) Zhang and colleagues described in this Research Report (Zhang et al. 2013) was funded later, under Request for Preliminary Applications (RFPA) 05-3, "Health Effects of Air Pollution."

This preface describes both the framework of outcomes research as it relates to air quality regulations and HEI's Outcomes Research program.

BACKGROUND

The first step in assessing the effectiveness of air quality regulations is to measure emissions of the targeted pollutants to see whether they have in fact decreased as intended. A series of intermediate assessments, described in detail below, are needed in order to accurately measure the adverse health effects associated with air pollution to see whether they, too, decreased in incidence or severity relative to emissions. Some outcomes studies to date have used hypothetical scenarios (comparing estimated outcomes under existing and more stringent regulations) and risk estimates obtained from epidemiologic studies in an attempt to quantify past effects on health and to predict future effects (U.S. EPA 1999). However, more extensive validation of these estimates with data on actual outcomes would be helpful.

The long-term improvements in U.S. air quality have been associated with improved health in retrospective epidemiologic studies (Chay and Greenstone 2003; Laden et al. 2006; Pope et al. 2009). Considerable challenges, however, are inherent in the assessment of

HEI's Outcomes Research Program: First-Wave Studies[a]

RFA / Investigator (Institution)	Study or Report Title	Intervention
RFA 02-1		
Douglas Dockery (Harvard School of Public Health, Boston, MA)	Effect of Air Pollution Control on Mortality and Hospital Admissions in Ireland (Research Report 176; in press)	Coal ban in Irish cities
Annette Peters (GSF–National Research Center for Environment and Health, Neuherberg, Germany[b])	The Influence of Improved Air Quality on Mortality Risks in Erfurt, Germany (published as Research Report 137, 2009)	Switch from brown coal to natural gas for home heating and power plants, changes in motor vehicle fleet after reunification of Germany
RFA 04-1		
Frank Kelly (King's College London, London, U.K.)	The Impact of the Congestion Charging Scheme on Air Quality in London: Part 1. Emissions Modeling and Analysis of Air Pollution Measurements. Part 2. Analysis of the Oxidative Potential of Particulate Matter (published as Research Report 155, 2011)	Measures to reduce traffic congestion in the inner city of London
RFA 04-4		
Frank Kelly (King's College London, London, U.K.)	The London Low Emission Zone Baseline Study (published as Research Report 163, 2011)	Measures to exclude most polluting vehicles from entering greater London
Richard Morgenstern (Resources for the Future, Washington, DC)	Accountability Analysis of Title IV Phase 2 of the 1990 Clean Air Act Amendments (published as Research Report 168, 2012)	Measures to reduce sulfur emissions from power plants east of the Mississippi River
Curtis Noonan (University of Montana, Missoula, MT)	Assessing the Impact of a Wood Stove Replacement Program on Air Quality and Children's Health (published as Research Report 162, 2011)	Wood stove change-out program
Jennifer Peel (Colorado State University, Fort Collins, CO)	Impact of Improved Air Quality During the 1996 Summer Olympic Games in Atlanta on Multiple Cardiovascular and Respiratory Outcomes (published as Research Report 148, 2010)	Measures to reduce traffic congestion during the Atlanta Olympics
Chit-Ming Wong (University of Hong Kong, Hong Kong)	Impact of the 1990 Hong Kong Legislation for Restriction on Sulfur Content in Fuel (published as Research Report 170, 2012)	Measures to reduce sulfur content in fuel for motor vehicles and power plants
RFPA 05-3		
Junfeng (Jim) Zhang (University of Medicine and Dentistry of New Jersey, Piscataway, NJ)	Cardiorespiratory Biomarker Responses in Healthy Young Adults to Drastic Air Quality Changes Surrounding the 2008 Beijing Olympics (published as Research Report 174, 2013)	Measures to improve air quality during the Beijing Olympics

[a] Abbreviations: RFA, Request for Applications; RFPA, Request for Preliminary Applications.

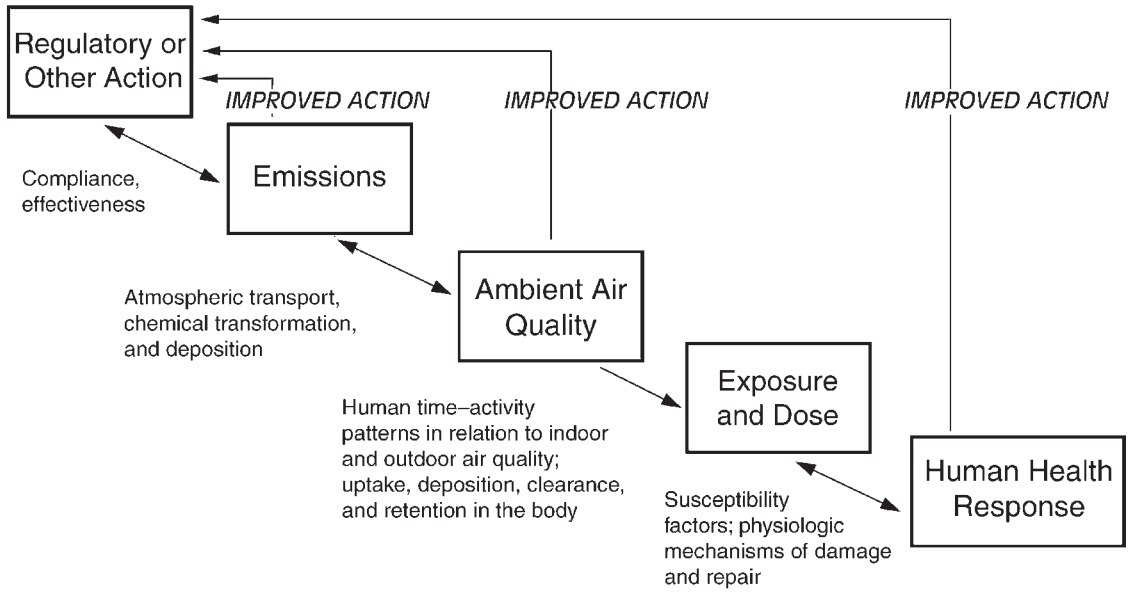
[b] As of 2008, this institution has been called the Helmholtz Zentrum München–German Research Center for Environmental Health.

the health effects of air quality regulations. Different regulations go into effect at different times, for example, and may be implemented at different levels of government (e.g., national, regional, or local). Their effectiveness therefore needs to be assessed in ways that take into account the varying times of implementation and levels of regulation. In addition, other changes at the same time and place might confound an apparent association between pollution reduction and improved health, such as economic trends (e.g., changes in employment), improvements in health care, and behavioral changes (e.g., staying indoors when government warnings indicate pollution concentrations are high). Moreover, adverse health effects that might have been caused by exposure to air pollution can also be caused by other environmental risk factors (some of which may have changed over the same time periods as the air pollution concentrations). These challenges become more pronounced when regulations are implemented over long periods and when changes in air quality and health outcomes are not seen immediately, thus increasing the chance for confounding by other factors. For these reasons, scenarios in which regulations are expected to have resulted in rapid changes in air quality tend to be among the first, and most likely, targets for investigation, rather than evaluations of complex regulatory programs implemented over multiple years. Studies in Ireland by Clancy and colleagues (2002) and in Hong Kong by Hedley and colleagues (2002) are examples of such scenarios.

These inherent challenges are well documented in Communication 11 (HEI 2003), which was intended to advance the concept of outcomes research and to foster the development of methods and studies throughout the relevant scientific and policy communities. In addition, recent advances in data collection and analytic techniques provide an unprecedented opportunity to improve our assessments of the effects of air quality interventions.

THE OUTCOMES EVALUATION CYCLE

The NRC's Committee on Research Priorities for Airborne Particulate Matter set out a conceptual framework for linking air pollution sources to adverse health effects (NRC 1998). This framework can be used to identify factors along an "outcomes evaluation cycle" (see Figure below), each stage of which affords its own opportunities for making quantitative measurements of the intended improvements.



Outcomes Evaluation Cycle. Each box represents a stage in the process between regulatory action and human health responses to air pollution. Arrows connecting the stages indicate possible directions of influence. The text below the arrows identifies factors affecting the effectiveness of regulatory actions at each stage. At several of the stages, knowledge gained from studies on outcomes can provide valuable feedback for improving regulatory or other actions.

At the first stage (regulatory action), one can assess whether controls on source emissions have in fact been put into place. At the second stage (emissions), one can determine whether controls on sources have indeed reduced emissions, whether emitters have changed their practices, and whether there have been unintended consequences. At the third stage (ambient air quality), one can assess whether controls on sources and reductions in emissions have resulted in improved air quality. At the fourth stage (personal or population exposure), one can assess whether the improvement in air quality has reduced people's actual exposure and whether susceptible subpopulations (those most likely to experience adverse health effects) have benefited. At this stage, it is important to take into account changes in time–activity patterns that could either increase or reduce exposure. The actual dose that an individual's organs may be exposed to should also be considered (i.e., whether reductions in exposure have led to reductions in concentrations in body tissues such as the lung). Finally, at the fifth stage (human health response), one can assess whether risks to health have declined, given the evidence about changes in health outcomes such as morbidity and mortality that have resulted from changes in exposure. The challenge at this stage is to investigate the health outcomes that are most directly related to exposure to air pollution.

At each stage in the outcomes evaluation cycle, the opportunity exists to collect evidence that either validates the assumptions that motivated the intervention or points to ways in which the assumptions were incorrect. The collection of such evidence can thus ensure that future interventions are maximally effective.

Ultimately, the framework for outcomes research will need to encompass investigations of the broader consequences of regulations, not just the intended consequences. Unintended consequences should also be investigated, along with the possibility that risks to public health in fact increased, as discussed by Wiener (1998) and others who have advanced the concept of a portfolio of effects of a regulation.

HEI'S OUTCOMES RESEARCH PROGRAM

The first wave of HEI's Outcomes Research program includes nine studies. The study by Dr. Junfeng (Jim) Zhang and colleagues presented in this report is the eighth to be published. The remaining study is in press and will be published in the spring of 2013.

These studies involve the measurement of indicators along the entire outcomes evaluation cycle, from regulatory or other interventions to human health outcomes. Some of the studies focused on interventions that are implemented over relatively short periods of time, such as a ban on the sale of coal, the replacement of old wood stoves with more efficient, cleaner ones, reductions in the sulfur content of fuels, and measures to reduce traffic. Other groups focused on longer-term, wider-ranging interventions or events; for instance, one study assessed complex changes associated with the reunification of the former East and West Germany, including a switch from brown coal to natural gas for fueling power plants and home-heating systems and an increase in the number of modern diesel-powered vehicles in eastern Germany. HEI is also supporting research, including the development of methods, in an especially challenging area, namely, assessment of the effects of regulations implemented incrementally over extended periods of time, such as those, examined in the study by Morgenstern et al. (2012), that resulted from Title IV of the 1990 Clean Air Act Amendments (U.S. EPA 1990), which aimed at reducing sulfur dioxide emissions from power plants by requiring compliance with prescribed emission limitations. Studies on health outcomes funded by HEI to date are summarized in the Table on page xii and described in more detail in an interim evaluation of the HEI Outcomes Research program (van Erp and Cohen 2009; van Erp et al. 2012).

FUTURE DIRECTIONS

As a part of its Strategic Plan for 2010 through 2015 (HEI 2010a), HEI has looked closely at opportunities for unique new contributions to health outcomes research. Key recommendations for future research were made at a December 2009 planning workshop (HEI 2010b), which led to HEI issuing a new RFA in January 2011 for a second wave of outcomes research. RFA 11-1, "Health Outcomes Research — Assessing the Health Outcomes of Air Quality Actions," solicited applications for studies designed to assess the health effects of actions to improve air quality and to develop methods required for, and specifically suited to, conducting such research. Recently, HEI approved four

studies: two will evaluate regulatory and other actions at the national or regional level implemented over multiple years; a third study will evaluate complex sets of actions targeted at improving air quality in large urban areas and major ports with well-documented air quality problems and programs to address them; and a fourth study will develop methods to support such health outcomes research. These studies are currently under way.

In addition, HEI has funded the development of two Web sites intended to enhance transparency and provide other researchers with access to extensive data and software from HEI-funded studies:

1. Data and software from the National Morbidity, Mortality, and Air Pollution Study (NMMAPS), as described by Zeger and colleagues (2006) (data available at the Johns Hopkins Bloomberg School of Public Health Web site *www.ihapss.jhsph.edu*); and
2. Data from the National Particle Component Toxicity (NPACT) initiative on concentrations of components of particulate matter with an aerodynamic diameter $\leq$ 2.5 μm ($PM_{2.5}$) collected at or near the 54 sites in the EPA's $PM_{2.5}$ Chemical Speciation Trends Network (STN) (data available at the Atmospheric and Environmental Research Web site *https://hei.aer.com*).

The data on pollution and health from a large number of U.S. cities, as documented by the NMMAPS team and made available on the Internet-Based Health and Air Pollution Surveillance System (iHAPSS) Web site, constitute a valuable resource that allows other researchers to undertake additional analyses, possibly including further outcomes studies. The STN Web site provides scientists an opportunity to investigate specific questions about concentrations of $PM_{2.5}$ components and their association with adverse health effects in regions covered by the STN network and to address questions related to outcomes research when interventions in these regions are being planned.

In January 2008, HEI co-organized and cosponsored, with the CDC's National Environmental Public Health Tracking Program and the EPA, a workshop titled "Methodologic Issues in Environmental Public Health Tracking of Air Pollution Effects." The workshop was part of an effort to implement the initiative outlined in HEI's Strategic Plan for 2005 through 2010 (HEI 2005) to "build networks with the U.S. Centers for Disease Control and Prevention and state public health tracking programs to facilitate accountability research."

The workshop built on the work of the CDC's National Environmental Public Health Tracking Program (see the CDC Web site *www.cdc.gov/nceh/tracking/*) in the development of standardized measures of air pollution–related effects on health at the state and local levels in the United States. It brought together representatives of state and federal agencies and academic researchers to discuss methodologic issues in developing standardized measures and made recommendations for their further development and application in assessing the health impacts of air pollution, including the impacts of actions taken to improve air quality. The recommendations were provided in a September 2008 report to the CDC, and the proceedings were published in the journal *Air Quality, Atmosphere & Health* in December 2009 (Matte et al. 2009). The CDC has subsequently funded a pilot project under the National Environmental Public Health Tracking Program to implement the recommendations of the workshop in selected states and metropolitan areas.

HEI will continue to seek opportunities to work with the CDC and the EPA to apply methods newly developed for tracking public health and assessing the effectiveness of environmental regulations.

Investigators who have identified a distinctive opportunity to evaluate the effects of environmental regulations on air pollution and human health are encouraged to contact HEI.

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HEI STATEMENT

Synopsis of Research Report 174

Health Impact of Changes in Air Pollution Levels and PM Composition Brought by the 2008 Olympic Games in Beijing

INTRODUCTION

HEI's Outcomes Research Program has been designed to evaluate the effects of regulatory and other actions taken to improve air quality. The overall goal has been to provide evidence about the extent to which air quality regulations may or may not have improved air quality and health. Some funded studies have looked at the effects of interventions lasting only a limited period of time. These studies took advantage of a unique event such as the 1996 Atlanta Olympics, for which traffic and other changes were to be made in the local area with the goal of improving air quality for the duration of the event. The current study capitalized on a similar unique event.

In 2006 Dr. Junfeng (Jim) Zhang, then of the University of Medicine and Dentistry of New Jersey–School of Public Health and the Environmental and Occupational Health Sciences Institute at Rutgers University, submitted a preliminary application to HEI, "Health Impact of Changes in Air Pollution Levels and PM Composition Brought by the 2008 Olympic Games in Beijing," in response to a request for preliminary applications issued by HEI. The investigator indicated that the Chinese government was launching a series of aggressive policies to reduce local and regional emissions that affected air quality in the greater Beijing metropolitan area in the period leading up to and during the 2008 Beijing Olympic Games. These controls aimed to limit both vehicular traffic on Beijing roads and emissions from industrial, power generation, and commercial facilities in Beijing, as well as construction activities. The goals of the study were to measure levels of air pollutants in the city and evaluate prospectively the impact on human cardiovascular responses of the likely changes in air pollution levels that would be associated with the control measures to be implemented. Zhang and colleagues hypothesized that levels of multiple cardiovascular biomarkers would change significantly during the Olympic air pollution reduction period compared with the pre-Olympics period, and would revert to pre-Olympics levels following relaxation of the air pollution controls after the Olympics. The investigators also hypothesized that changes in specific pollutants would be associated with changes in specific biomarkers.

APPROACH

Zhang and colleagues divided the study into three periods: the *pre-Olympics period* (June 2–July 20, 2008), the *during-Olympics period* (July 21–September 19), and the *post-Olympics period* (September 20–October 30). They made daily measurements of multiple air pollutants on the roof of a seven-story building located in the center of the Peking University First Hospital campus: mass concentration of particles less than or equal to 2.5 μm in aerodynamic diameter ($PM_{2.5}$), elemental carbon (EC) and organic carbon (OC), inorganic ions (including sulfate and nitrate), polycyclic aromatic hydrocarbons, and trace elements (including multiple transition metals such as nickel and vanadium). The investigators also measured levels of multiple gaseous pollutants — ozone (O_3), carbon monoxide (CO), sulfur dioxide (SO_2), nitric oxide (NO), nitrogen dioxide (NO_2), nitrous oxides (NO_x) — as well as ambient temperature and relative humidity at the central site. As a result of technical problems with the instrument at the central site, the investigators had to rely on a site 7 km from where the other pollutant measurements were made for

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr. Jim Zhang at the University of Medicine and Dentistry of New Jersey–School of Public Health, Piscataway, N.J., and colleagues. Research Report 174 contains both the detailed Investigators' Report and a Commentary on the study prepared by the Institute's Health Review Committee.

most measurements of the number of ultrafine particles (<100 nm in aerodynamic diameter) — that is, particle number concentration and total particle number (TPN).

The investigators reported results from 125 healthy young (ages 19–33) subjects — primarily medical residents who worked at Peking First University Hospital in Central Beijing and who lived within 5 km of the Hospital. At each of six clinical visits — two within each period (and separated by at least 1 week) — vital signs and a set of biomarkers were measured in each study subject. The endpoints measured included electrocardiography (ECG), heart rate (HR) and heart rate variability (HRV), systolic (SBP) and diastolic (DBP) blood pressure; biomarkers of systemic inflammation and oxidative stress, including white blood cell (WBC) counts and differential cell counts in plasma, as well as levels of fibrinogen, C-reactive protein (CRP), and urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG); biomarkers of pulmonary inflammation and oxidative stress: fractional exhaled nitric oxide (FeNO), and exhaled breath condensate (EBC) — specifically, pH (from which they calculated hydrogen ion concentration), nitrate, nitrite, nitrite+nitrate, and 8-isoprostane; and biomarkers of plasma clotting pathways: platelet activation (soluble P-selectin [sCD62P] and soluble CD40 ligand [sCD40L]), platelet aggregation, and von Willebrand factor [vWF]).

The investigators used two approaches to analyze associations between pollutant levels and most biomarkers — mixed models to examine the effects of period (pre-, during-, and post-Olympics), and a time-series analysis to examine pollutant effects, focusing on changes within the few days prior to a clinical visit (lag days 0–6, where lag day 0 is the day of a visit). Zhang and colleagues also conducted multiple sensitivity analyses for the associations between biomarkers and pollutant levels.

RESULTS

Compared with the pre-Olympics period, the mean during-Olympics concentrations of all measured pollutants, except O_3, decreased: large decreases (40–60%) were found for the pollutant gases SO_2, CO, and NO_2, and particulate pollutants ($PM_{2.5}$ and its components EC and TPN) also decreased substantially. Smaller changes were found in sulfate and OC concentrations. The decreases in concentration of SO_2, CO, and NO_2 were statistically significant, but the reductions in mean concentrations of $PM_{2.5}$, sulfate, EC, TPN, and OC were not. By contrast, O_3 concentrations increased during the Olympics. Compared with the during-Olympics period, post-Olympics concentrations of most pollutants increased, except for sulfate and O_3, which decreased.

During-Olympics levels of several cardiovascular markers decreased compared with pre-Olympics levels. The biggest percent decreases were observed in FeNO (60.3%), 8-OHdG (58.3%), EBC pH (which increased 3.5%, but corresponded to a decrease in hydrogen ion concentration of 46%), sCD62P (34%), EBC nitrite (30.0%), EBC nitrate (21.5%), EBC nitrite+nitrate (17.6%), and vWF (13.1%). Smaller decreases were also seen in HR (1.7%), SBP (1.8%), and levels of sCD40L (5.7%). Unexpected small increases in platelet aggregation (7.4%) and RBC numbers (0.9%) were also found. No significant changes were found in any HRV measurement or DBP. After the Olympics, concentrations of most biomarkers increased (after decreasing in the during-Olympics period).

INTERPRETATION AND CONCLUSIONS

In its independent review, the HEI Health Review Committee considered the study an important contribution to the literature regarding short-term interventions and their impacts on acute health responses — it is one of the first studies, and to date the most comprehensive, to evaluate changes in biologic endpoints associated with the control measures taken to reduce air pollution associated with specific, short-term events. The investigators capitalized on the large changes in air pollutants to conduct an analysis by period (pre-, during-, and post-Olympics) to assess whether biomarkers were associated with those changes. A more traditional time-series analysis, focusing on very proximate (within a few days) pollution–biomarker associations, gave a somewhat complementary perspective of the data. The exposure assessment for multiple pollutants was also relatively comprehensive. Apart from measurements of particle number, the measurements of all pollutants were made at a single central Beijing site, close to where the participants lived and worked. Furthermore, in this well-characterized group of healthy subjects, Zhang and colleagues evaluated a representative group of pulmonary, systemic, and urinary biomarkers in pathways considered relevant for understanding the pathophysiologic mechanisms of the effects of air pollutants. Zhang and colleagues also conducted

appropriate sensitivity analyses — including adjusting for meteorology and (in the time-series analyses) for an effect of period independent of pollution — to provide further support for their interpretations. Thus, the study represents one of the most comprehensive to date to evaluate the effects of exposure to air pollution on a myriad of potential short-term cardiovascular biomarkers.

Reviewing Zhang and colleagues' comparisons of pollutant concentrations before and during the Olympics, the Committee agreed that the investigators found many during-Olympics changes in pollutant levels and that these were consistent with the effects of a successful intervention. However, the Committee noted that the investigators had not designed the study to identify the extent to which the control measures per se could be considered causal in producing the changes in ambient pollutant levels. Therefore, the changes in the biomarkers they had measured, which were consistent with the measured improvements of air quality, may not be directly attributed to the interventions.

The large during-Olympics decreases (40–60%) found for the pollutant gases SO_2, CO, and NO_2, and for particulate pollutants (EC and TPN, with smaller changes in $PM_{2.5}$, SO_4^{2-}, and OC concentrations) were generally consistent with other studies of pollutant concentrations in Beijing conducted in the time period around the Olympics. The major decrease in during-Olympics concentrations of SO_2 was most likely due to the restrictions imposed on construction and power plant activities in and around Beijing. Decreases in NO_2, CO, EC, and TPN (the last a marker of ultrafine particles, which dominate this measure) were most likely attributable to restrictions on traffic. After the controls were relaxed in the post-Olympics period, concentrations of most pollutants (except O_3) increased, and changes were seen in PM components that were generally consistent with increases in traffic and other emissions sources.

Changes in the levels of several cardiovascular biomarkers from the pre- to during-Olympics periods were observed, and the Committee agreed with Zhang and colleagues that these changes were generally consistent with the investigators' hypothesis that changes would be in a direction reflecting a decrease in adverse effects. Using both by-period and time-series analyses, the investigators found large changes in some biomarkers in several pathophysiologic pathways through which PM may exert its effects — coagulation in the circulation (sCD62P and vWF), inflammation in the airways (FeNO, EBC hydrogen ion, nitrite, and nitrate), and the activation of oxidative stress (urinary 8-OHdG). However, unexpectedly and without obvious explanation, levels of some biomarkers — in particular platelet aggregation in the coagulation pathway — changed in the opposite direction from other biomarkers in the same pathway. Given the number of observations made in the study, a few might have increased by chance.

The finding that the post-Olympics mean levels of several biomarkers increased compared with during-Olympics levels was largely consistent with the investigators' hypotheses that changes in air pollutant levels in the different periods would be reflected in changes in biomarker levels and that the pre- to during- and during- to post-Olympics changes would be inversely related. It is noteworthy that these observations were made in young healthy subjects and so may not reflect changes that might be seen in susceptible populations, such as those with pre-existing cardiorespiratory conditions (e.g., asthma or cardiovascular disease) or those with variations in genes whose protein products are involved in physiologic defenses. In addition, given the study's focus on acute reversible changes after exposure to air pollution, this study does not shed light on whether these changes would have any impact, positive or negative, on disease or adverse outcomes.

Although the investigators had hypothesized that individual pollutants would be associated with changes in individual biomarkers, multiple pollutants were associated with every biomarker. In hindsight, attributing changes to specific pollutants was likely to be challenging, given that the intervention was multifaceted and affected multiple sources and pollutants.

The multiple sensitivity analyses Zhang's team conducted bolstered the interpretation that the changes in biomarkers were related to changes in levels of air pollution. However, the Committee thought that some caution should be retained in attributing the between-period biomarker differences to pollution, given the possibility that other unmeasured risk factors — such as changes in virus infections, ambient noise levels, or subtle alterations in lifestyle patterns and stress levels in the participants in response to the atmosphere surrounding the Olympics — might have contributed to the differences observed. Because of the large influence of

between-period contrasts in the time-series analyses of the association of specific pollutants with biomarkers, similar caution is needed for these results. The time-series analyses were generally consistent with the between-period analyses, but could not discriminate among the several pollutants, which changed in concert. Exposure misclassification may also have been an issue — that is, pollutant measurements made at the outdoor site may not have accurately reflected the actual level of exposure of the study participants. However, the Committee considered that it was unlikely there was a systematic pattern to this type of error, and so this possible error was considered unlikely to have an impact on the effect estimates reported in the current study.

The Committee suggested that future studies to evaluate the effects of an intervention in a city should include a nearby control area — as similar in characteristics to the area of the intervention as possible — in which exposure would be measured and a similar group of participants would be followed with the same instruments over the same time period. Although the cost of the study would increase substantially, the Committee thought inclusion of this control would enhance the investigators' ability to attribute the changes in pollutant levels and biomarkers to the intervention and greatly reduce the limitations of the interpretation of the results as noted above.

Overall, this study carried out by Zhang and colleagues provides important supporting evidence that air quality improvements such as those found during the Beijing Olympics can improve health biomarkers, with the potential for beneficial health effects in the affected population.

INVESTIGATORS' REPORT

Cardiorespiratory Biomarker Responses in Healthy Young Adults to Drastic Air Quality Changes Surrounding the 2008 Beijing Olympics

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ABSTRACT

Associations between air pollution and cardiorespiratory mortality and morbidity have been well established, but data to support biologic mechanisms underlying these associations are limited. We designed this study to examine several prominently hypothesized mechanisms by assessing Beijing residents' biologic responses, at the biomarker level, to drastic changes in air quality brought about by unprecedented air pollution control measures implemented during the 2008 Beijing Olympics.

To test the hypothesis that changes in air pollution levels are associated with changes in biomarker levels reflecting inflammation, hemostasis, oxidative stress, and autonomic tone, we recruited and retained 125 nonsmoking adults (19 to 33 years old) free of cardiorespiratory and other chronic diseases. Using the combination of a quasi-experimental design and a panel-study approach, we measured biomarkers of autonomic dysfunction (heart rate [HR*] and heart rate variability [HRV]), of systemic inflammation and oxidative stress (plasma C-reactive protein [CRP], fibrinogen, blood cell counts and differentials, and urinary 8-hydroxy-2′-deoxyguanosine [8-OHdG]), of pulmonary inflammation and oxidative stress (fractional exhaled nitric oxide [FeNO], exhaled breath condensate [EBC] pH, EBC nitrate, EBC nitrite, EBC nitrite+nitrate [sum of the concentrations of nitrite and nitrate], and EBC 8-isoprostane), of hemostasis (platelet activation [plasma sCD62P and sCD40L], platelet aggregation, and von Willebrand factor [vWF]), and of blood pressure (systolic blood pressure [SBP] and diastolic blood pressure [DBP]). These biomarkers were measured on each subject twice before, twice during, and twice after the Beijing Olympics. For each subject, repeated measurements were separated by at least one week to avoid potential residual effects from a prior measurement. We measured a large suite of air pollutants ($PM_{2.5}$ [particulate matter $\leq$ 2.5 μm in aerodynamic diameter] and constituents, sulfur dioxide [SO_2], carbon monoxide [CO], nitrogen dioxide [NO_2], and ozone [O_3]) throughout the study at a central Beijing site near the residences and workplaces of the subjects on a daily basis. Total particle number (TPN) was also measured at a separate site. We used a time-series analysis to assess changes in pollutant concentration by period (pre-, during-, and post-Olympics periods). We used mixed-effects models to assess changes in biomarker levels by period and to estimate changes associated with increases in pollutant concentrations, controlling for ambient temperature, relative humidity (RH), sex, and the day of the week of the biomarker measurements. We conducted

This Investigators' Report is one part of Health Effects Institute Research Report 174, which also includes a Commentary by the Health Review Committee and an HEI Statement about the research project. Correspondence concerning the Investigators' Report may be addressed to Dr. Junfeng Zhang, University of Southern California, Keck School of Medicine, 2001 N. Soto Street, Room 225Q, Los Angeles, CA 90089; email: *junfengz@usc.edu*

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* A list of abbreviations and other terms appears at the end of the Investigators' Report.

sensitivity analyses to assess the impact of potential temporal confounding and exposure misclassification.

We observed reductions in mean concentrations for all measured pollutants except O_3 from the pre-Olympics period to the during-Olympics period. On average, elemental carbon (EC) changed by −36%, TPN by −22%, SO_2 by −60%, CO by −48%, and NO_2 by −43% ($P < 0.05$ for all these pollutants). Reductions were observed in mean concentrations of $PM_{2.5}$ (by −27%), sulfate (SO_4^{2-}) (by −13%), and organic carbon (OC) (by −23%); however, these values were not statistically significant. Both 24-hour averages and 1-hour maximums of O_3 increased (by 20% and 17%, respectively) from the pre-Olympics to the during-Olympics period. In the post-Olympics period after the pollution control measures were relaxed, mean concentrations of most pollutants (with the exception of SO_4^{2-} and O_3) increased to levels similar to or higher than pre-Olympics levels.

Concomitantly and consistent with the hypothesis, we observed, from the pre-Olympics to the during-Olympics period, statistically significant ($P \leq 0.05$) or marginally significant ($0.05 < P < 0.1$) decreases in HR (−1 bpm or −1.7% [95% CI, −3.4 to −0.1]), SBP (−1.6 mmHg or −1.8% [95% CI, −3.9 to 0.4]), 8-OHdG (−58.3% [95% CI, −72.5 to −36.7]), FeNO (−60.3% [95% CI, −66.0 to −53.6]), EBC nitrite (−30.0% [95% CI, −39.3 to −19.3]), EBC nitrate (−21.5% [95% CI, −35.5 to −4.5]), EBC nitrite+nitrate (−17.6% [95% CI, −28.4 to −5.1]), EBC hydrogen ions (−46% [calculated from EBC pH], or +3.5% in EBC pH [95% CI, 2.2 to 4.9]), sCD62P (−34% [95% CI, −38.4 to −29.2]), sCD40L (−5.7% [95% CI, −10.5 to −0.7]), and vWF (−13.1% [95% CI, −18.6 to −7.5]). Moreover, the percentages of above-detection values out of all observations were significantly lower for plasma CRP and EBC 8-isoprostane in the during-Olympics period compared with the pre-Olympics period. In the post-Olympics period, the levels of the following biomarkers reversed (increased, either with or without statistical significance) from those in the during-Olympics period: SBP (10.7% [95% CI, 2.8 to 18.6]), fibrinogen (4.3% [95% CI, −1.7 to 10.2), neutrophil count (4.7% [95% CI, −7.7 to 17.0]), 8-OHdG (315% [95% CI, 62.0 to 962]), FeNO (130% [95% CI, 62.5 to 225]), EBC nitrite (159% [95% CI, 71.8 to 292]), EBC nitrate (161% [95% CI, 48.0 to 362]), EBC nitrite+nitrate (124% [95% CI, 50.9 to 233]), EBC hydrogen ions (146% [calculated from EBC pH] or −4.8% in EBC pH [95% CI, −9.4 to −0.2]), sCD62P (33.7% [95% CI, 17.7 to 51.8]), and sCD40L (9.1% [95% CI, −3.7 to 23.5]).

Furthermore, these biomarkers also showed statistically significant associations with multiple pollutants across different lags after adjusting for meteorologic parameters. The associations were in the directions hypothesized and were consistent with the findings from the comparisons between periods, providing further evidence that the period effects were due to changes in air quality, independent of season and meteorologic conditions or other potential confounders. Contrary to our hypothesis, however, we observed increases in platelet aggregation, red blood cells (RBCs) and white blood cells (WBCs) associated with the during-Olympics period, as well as significant negative associations of these biomarkers with pollutant concentrations. We did not observe significant changes in any of the HRV indices and DBP by period. However, we observed associations between a few HRV indices and pollutant concentrations.

Changes in air pollution levels during the Beijing Olympics were associated with acute changes in biomarkers of pulmonary and systemic inflammation, oxidative stress, and hemostasis and in measures of cardiovascular physiology (HR and SBP) in healthy, young adults. These changes support the prominently hypothesized mechanistic pathways underlying the cardiorespiratory effects of air pollution.

INTRODUCTION

Epidemiologic evidence documents that daily changes in ambient concentrations of air pollutants, particularly but not exclusively PM, are associated with variations of one to a few percent in both mortality and morbidity from cardiovascular and respiratory causes (Brook et al. 2004; Brook 2008). However, specific biologic mechanisms for these outcomes remain poorly understood. Although differences in mortality and morbidity outcomes are also observed when making spatial comparisons (i.e., comparing outcomes associated with chronic exposures between geographic regions or cities) (Chen et al. 2008; Puett et al. 2009), they are especially prominent when making temporal comparisons (changes over time within the same population or the same city). Many studies demonstrate statistically significant variation in short-term (daily) rates of cardiopulmonary outcomes such as myocardial infarction and heart failure exacerbations (Peters et al. 2004; Wellenius et al. 2006; Brook 2008). Thus, exploration of the mechanisms underlying cardiorespiratory responses that occur within an acute to subchronic time frame of air pollution exposure is clearly warranted. The present study focuses on short-term variations in air pollution concentrations in order to elucidate biologic mechanisms by which air pollution acutely triggers biochemical and physiologic events underlying clinically observed outcomes.

The study was designed to examine several prominent hypotheses regarding biologic pathways linking air pollution exposure and adverse cardiorespiratory outcomes within a time window of 1 to 7 days.

These interrelated pathways have been proposed to explain the epidemiologic phenomena of air-pollution-induced acute mortality and morbidity; for most of these hypothetical pathways, there is some corroborating, but inconclusive, evidence. A diagram that summarizes the hypothesized effects of air pollution on various pathways is shown in Figure 1, along with relevant biomarkers for each endpoint chosen for the present study. Inhalation of air pollutants, especially fine particles ($PM_{2.5}$ and its constituents), is now widely considered to induce inflammation in the respiratory tract and probably systemically, as demonstrated in previous studies linking air pollution exposure with one or more of the inflammatory biomarkers used in the present study (Salvi et al. 1999; Churg and Brauer 2000; Liao et al. 2004; Chuang et al. 2007; Eilstein 2009; O'Neill et al. 2009). The biomarkers selected for the present study are either clinically validated or established within the research field. For example, FeNO, produced primarily in the lung by inducible nitric oxide synthase, is an established clinical marker of pulmonary inflammation prevalent in asthmatic patients (Artlich et al. 1996; van Amsterdam et al. 2000). EBC nitrite and nitrate result from metabolic oxidation of nitric oxide in the lung (Hunt et al. 2000). A decrease in EBC pH has been associated with asthma exacerbation (McCreanor et al. 2007) and bronchoconstriction (Ricciardolo et al. 1999). In the liver, inflammatory mediators can lead to increased production of acute phase proteins in plasma such as CRP and fibrinogen (Slaughter et al. 2003). Plasma vWF, an adhesive glycoprotein produced by endothelial cells that allows platelets to attach to the sub-endothelial vessel wall, is an endothelial-derived coagulation marker and is also linked to systemic inflammation (Zezos et al. 2005). In addition, blood cell counts and differentials (e.g., WBCs, RBCs, and neutrophils) may serve as markers of cardiovascular disease risk (Seaton et al. 1995) and are often linked to inflammatory processes (Liao et al. 2005).

Air pollution exposure is also thought to affect autonomic tone — altering sympathetic and parasympathetic output, possibly affecting the stability of atherosclerotic

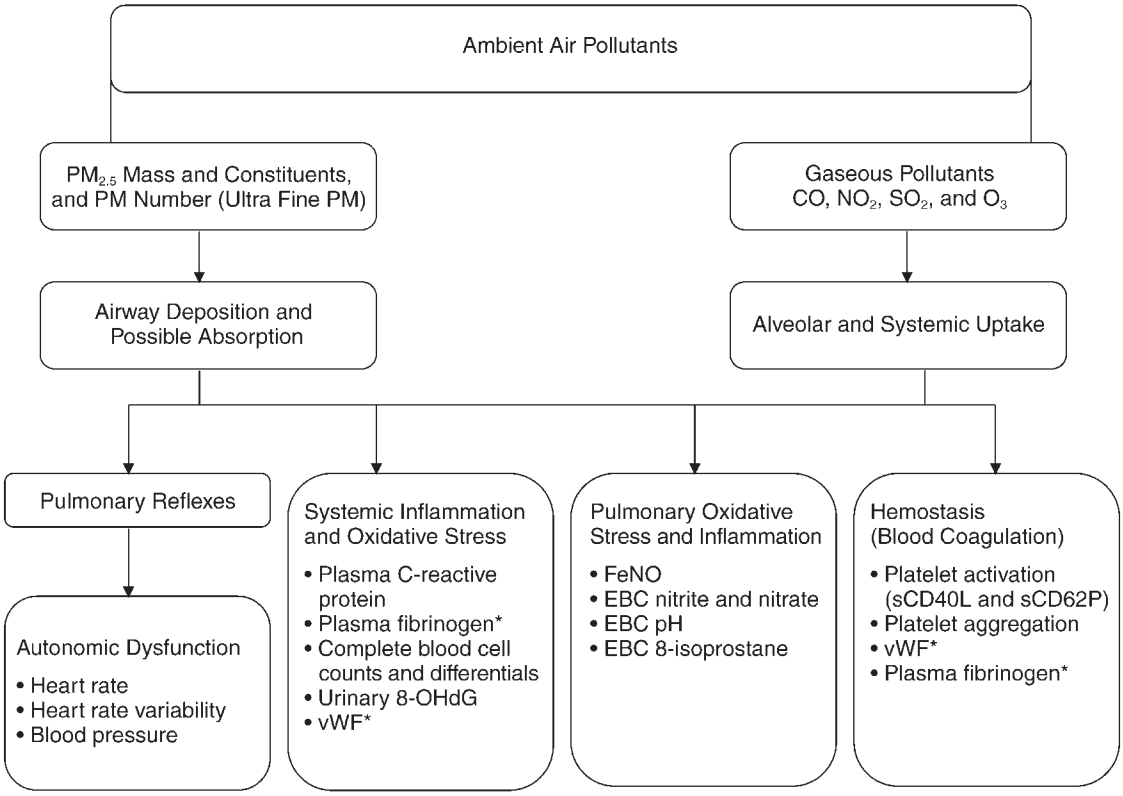

Figure 1. Diagram showing hypothesized pathways of the effects of air pollution on cardiorespiratory endpoints and biomarkers measured in the study. The asterisk (*) indicates biomarkers reflecting a secondary (overlapping) pathway.

plaques, and possibly decreasing HRV (Creason et al. 2001; Magari et al. 2002; Schwartz et al. 2005; Chuang et al. 2007). Decreased HRV is a suggested risk factor for arrhythmia and other causes of cardiovascular mortality (Liao et al. 1997). BP and HR are well-established risk factors for cardiovascular health (Reil and Böhm 2007). Other potential mechanisms include acute changes in vascular or endothelial function (Peretz et al. 2008) and the tendency for arterial thrombosis to develop, either due to platelet alterations (e.g., platelet aggregation) or changes in the soluble clotting system (Lucking et al. 2008). Two specific soluble markers (soluble CD40 ligand [sCD40L] and soluble P-selectin [sCD62P]) measured in the present study are released by platelets and are considered indicators of platelet activation, a precursor of blood coagulation, or hemostasis. Fibrinogen is an acute phase reactant synthesized by the liver and is vital to platelet aggregation. Like vWF, plasma fibrinogen is also related to systemic inflammation (see Figure 1).

Air pollution is likely to induce oxidative stress, which may also lead to health effects (Xia et al. 2006). Oxidative stress is hypothesized to be a primary mechanistic link between environmental or pathologic stimuli and inflammation, although the inflammatory response itself can produce oxidative stress through the release of cellular mediators. These oxidative-stress-related responses may occur in those tissues directly exposed to air pollutants (the respiratory tract) as well as systemically, presumably through inflammatory mechanisms or transport of metals and other toxicants from the alveoli (Sørensen et al. 2003; Delfino et al. 2008). In the present study, we used EBC 8-isoprostane as a primary biomarker of pulmonary oxidative stress, because it is a stable product of lipid peroxidation induced by reactive oxygen species (ROS) (Liu et al. 2009). We also considered EBC nitrite and nitrate to be pulmonary oxidative stress markers (as well as inflammatory markers) because ROS presumably affect the oxidation of nitric oxide (NO) in the lung (Kostikas et al. 2002). Finally, we selected 8-OHdG excreted in the urine as a systemic oxidative stress marker, because it is a stable compound produced by ROS oxidative reactions with DNA molecules (Kadiiska et al. 2005).

Among various types of studies examining one or more of these hypothetical biologic pathways (including in vitro studies, in vivo animal studies, and human "exposure chamber" or field studies), panel studies have been used frequently because they have a number of design advantages (Ruckerl et al. 2006; Delfino et al. 2008). The core feature of the panel-study design is the use of a smaller, but more intensively sampled, cohort. In this design, a group of individual subjects is followed longitudinally forward in time, allowing serial collection of health endpoint data, usually analyzed as a function of daily changes in ambient levels of pollution. While some panel studies collect symptom or other self-report information, most collect specimens of blood, breath, urine, or other biologic samples for laboratory analysis. Panels are typically smaller than cohort or case-control designs, using tens, rather than hundreds or thousands, of subjects. Most panel studies of the health effects of air pollution use typical day-to-day (or season-to-season) variation in ambient pollutant levels (Chuang et al. 2007), although this design has occasionally been modified to incorporate the more substantial pollution changes that accompany natural disasters (Peters et al. 1997; Tan et al. 2000). Planned interventions, on the other hand, could provide a more ideal opportunity for a prospective panel study, because air pollution changes may be predicted in advance, allowing for a more informed study design. A few epidemiologic studies of air pollution and health effects have taken advantage of "real-world experiments" resulting from a regulatory action, large-scale sporting event, or the closing and reopening of an industrial facility for an extended period that led to a substantial reduction in ambient air pollution levels (Heinrich et al. 2000; Clancy et al. 2002; Hedley et al. 2002; Pope et al. 2007; Parker et al. 2008; Peel et al. 2010). A common feature of planned air quality intervention programs is that a ban on a particular air pollution source, over a relatively short time period, results in sharp, substantial, and sustained reductions in ambient pollution. Associated declines in morbidity, such as asthma events and bronchitis, ranged up to 42%, while declines in mortality were generally more modest, on the order of 2% to 15% over longer time periods (Pope 1989; Heinrich et al. 2000; Clancy et al. 2002; Hedley et al. 2002; Lee et al. 2006; Pope et al. 2007; Parker et al. 2008; Peel et al. 2010). None of these intervention studies, however, has assessed biomarkers reflecting mechanisms for pollution-induced clinical events.

Beijing is one of the most polluted megacities in the world, with annual mean concentrations of $PM_{2.5}$ exceeding 100 μg/m^3 (Zhang et al. 2010) and daily mean concentrations of $PM_{2.5}$ at times exceeding 200 μg/m^3 (Xu and Zhang 2004). As one of its commitments to win the bid to host the 2008 Olympic and Paralympic Games, the Chinese government used its authority to control air pollution through specific actions, summarized in Figure 2, in order to ensure that the ambient air quality in Beijing during the Games would be comparable to that of previous host cities.

The temporary air quality improvements resulting from the air pollution control measures shown in Figure 2 provided a rare opportunity to address critical questions

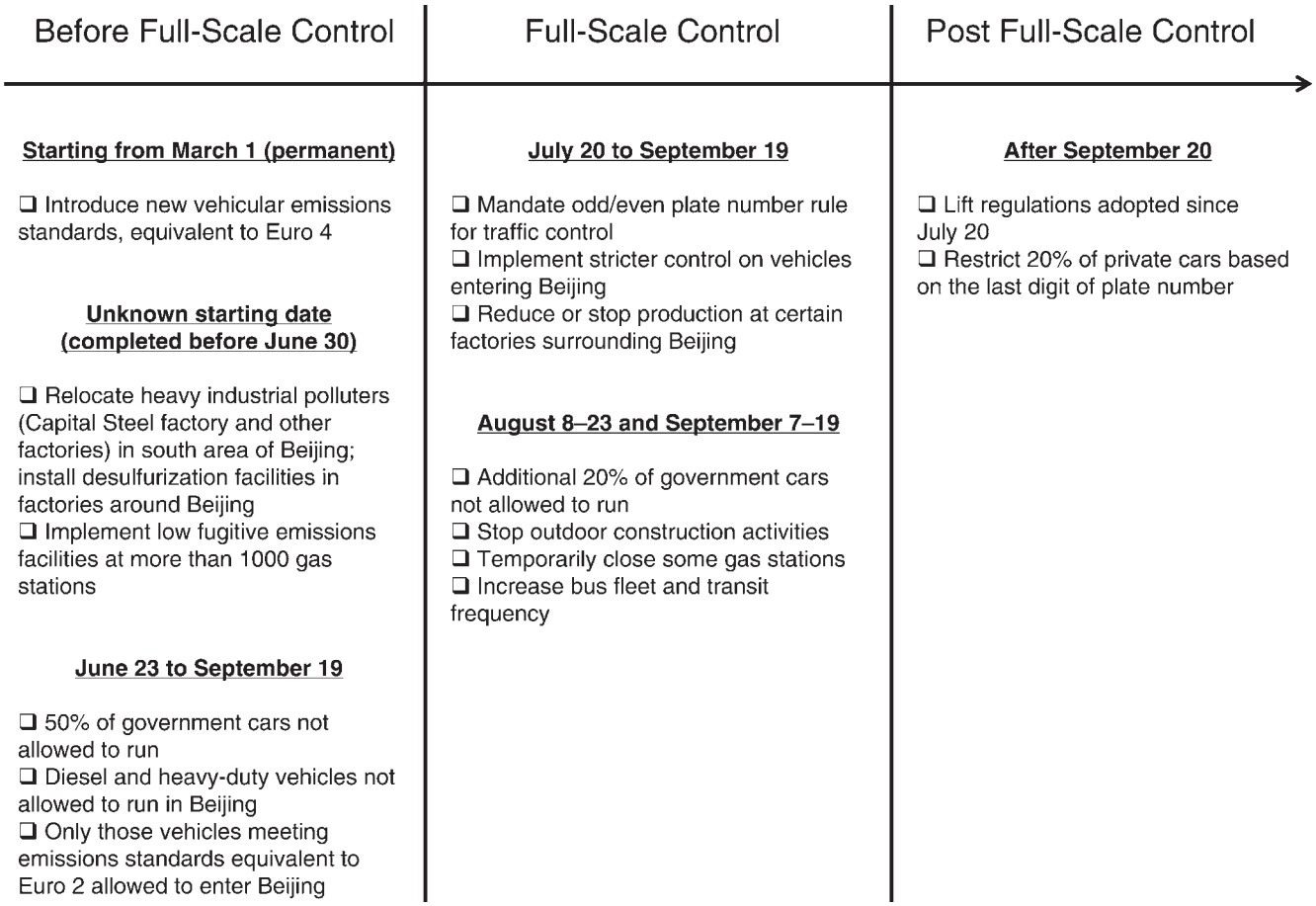

Figure 2. Air pollution control measures implemented to improve Beijing's air quality during the Olympics and Paralympics. (Data from Wang et al. 2009.)

regarding acute biologic mechanisms of cardiovascular effects related to ambient pollution over a uniquely broad concentration range. Such natural experiments also provide a rare opportunity for a quasi-experimental, "high-low-high" (or "A-B-A") design where exposures and outcomes can be measured at a baseline (A), then measured after a dramatic change in pollution levels (B), and finally measured again after an expected return to baseline exposure conditions (A). Hence, in the present study, named the "Health Effects of an Air Pollution Reduction Trial" (or the HEART study), we used a combination of the quasi-experimental design and a panel-study approach to address the following specific aims.

SPECIFIC AIMS

Aim 1. To estimate the exposure of the study panel (subjects) to ambient air pollution before, during, and after the Olympics and to quantify the change in exposure to each pollutant from before to during the Olympics; additionally, to examine whether the post-Olympics pollutant concentrations would return to pre-Olympics levels.

Aim 2. To examine the reversibility of changes in biomarker levels in the study panel by comparing level changes from before to during the Olympics and from during to after the Olympics. We aimed to test the following hypothesis: *Biomarkers of pulmonary inflammation, systemic inflammation, blood coagulation including platelet activation, and autonomic dysfunction, as well as biomarkers of oxidative stress, would change significantly during the Olympics air pollution reduction period, compared with the pre-Olympics period, and would revert after relaxation of the air pollution controls in the post-Olympics period.*

Aim 3. To examine associations between each biomarker and each measured pollutant species across the entire study period and to estimate the unit change in biomarker level per unit concentration change in pollutant

species. We aimed to test the following hypothesis: *$PM_{2.5}$, gaseous pollutants, and certain PM constituents would each be associated with specific biomarkers.*

METHODS AND STUDY DESIGN

This panel study was designed taking into account the timeline of the 2008 Beijing air pollution control measures as shown in Figure 2. As shown in Figure 3, the whole study period (June 2–October 30, 2008) was divided into three subperiods, namely, the pre-Olympics period (June 2–July 20), the during-Olympics period (July 21–September 19), and the post-Olympics period (September 20–October 30). A set of biomarkers was repeatedly measured in each panel subject during his or her six scheduled clinical visits: two within each subperiod, separated by at least one week. Air pollution measurements were made on a daily basis, either continuously for the whole study period or starting 7 days before the first day of clinical visits within each of the pre- and post-Olympics periods (see Figure 3).

We selected this relatively narrow time window (~5 months) for the study to minimize potential seasonal confounding of the health effects of air pollution exposure. In Beijing, the official season during which central heating is provided to most homes and residents may heat their homes (as determined by the government) is normally from November 15 to March 15, and regionally transported dust storms typically occur in early spring (before May 1), both circumstances that could effect air quality. According to our measurement scheme, visits 1 through 4 occurred in the summer months and visits 5 and 6 occurred in the early autumn season. Hence, meteorologic conditions during the two post-Olympics visits (especially those that occurred in late October) were expected to be different from those occurring during the first four visits.

This study design allowed us to compare within-subject differences in biomarker levels among the three subperiods and to assess relationships between each biomarker and each air pollutant across the study period. Moreover, a design incorporating repeated measurements

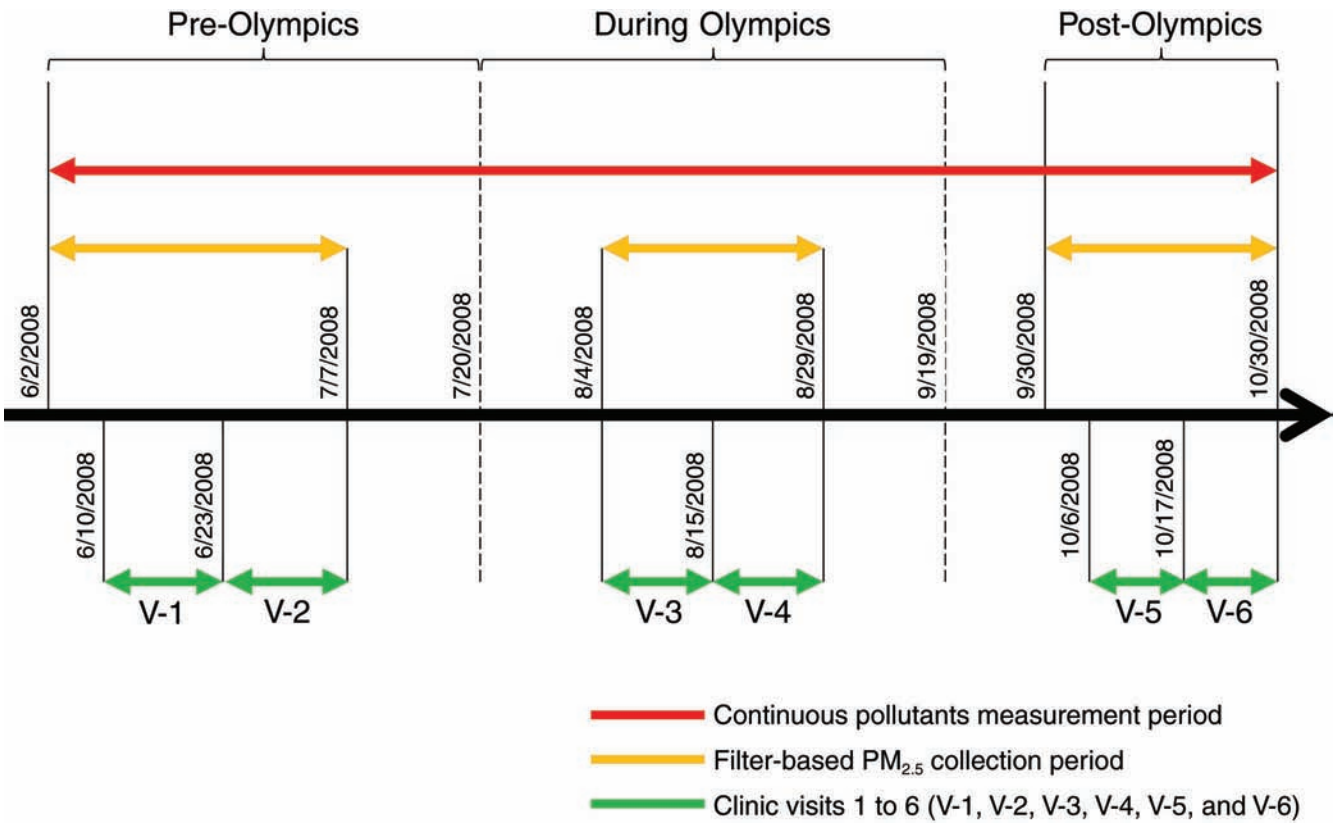

Figure 3. Operational definition of pre-, during-, and post-Olympics periods in relation to the start and end dates for air pollutant measurements and clinical visits within each period.

within each period would help reduce the impact of the potential variability of biomarkers within periods and between individuals.

FIELD STUDY SITE

We selected Peking University First Hospital as the field site for both the air pollution measurements and the clinical visits. The hospital is located in the center of Beijing (within the 2nd Ring Road, one of the main circular roads surrounding the city), 3 km northwest of Tiananmen Square; it is surrounded by busy streets with local motor vehicle traffic, cyclists, and pedestrians. As an institution affiliated with Peking University, the hospital has multiple functions, including teaching, clinical service, research, and disease prevention. At the time of the study, the hospital had more than 300 medical residents who were, after having completed a high school degree, in an 8-year medical education program; the sixth- through eighth-year students were receiving clinical training.

STUDY SUBJECTS

Our study subjects were primarily medical residents. Some of these residents (8%) lived in dormitories located on the hospital grounds, and the rest (84%), with a few exceptions, lived in the Peking University Health Sciences Center dormitories, located about 5 km from the hospital. These medical residents had a regimented lifestyle, eating at the hospital/university dining facilities, living in dormitories (with no cooking facilities and thus without a major indoor source of air pollution), attending lectures in classrooms, studying in libraries, and working in the hospital. A few subjects (8%) lived off campus in nearby areas.

Through on-site advertisement and word of mouth, we recruited volunteers from the subject pool with the goal of having at least 100 participants (with approximately 50% each of men and women) to complete measurements at all of the six planned clinical visits. A total of 137 individuals were screened, from whom 128 nonsmoking healthy subjects (never-smokers) were enrolled in the study. The other 9 either did not meet the inclusion criteria or refused to participate. To be eligible as a study subject, at a minimum an individual must not have smoked for at least the past year and be free of any of the following diseases: chronic respiratory disease, cardiovascular disease, liver disease, renal disease, hematologic disease, diabetes mellitus, and other systemic diseases. After taking part in the first one or two visits, 3 of the 128 subjects withdrew from the study because of scheduling conflicts. These 3 subjects were excluded from the data analysis. Among the 125 subjects included in the analysis, 119 completed all of the 6 planned visits, and the remaining 6 subjects missed only 1 visit. All the subjects were ethnic Han Chinese. Basic demographic information for the 125 study subjects is summarized in Table 1.

ETHICS AND INSTITUTIONAL REVIEW BOARD APPROVAL

The HEART study involved human subjects and was carried out at two collaborating institutions: the University of Medicine and Dentistry of New Jersey (UMDNJ) and Peking University in Beijing. The study protocol was approved by both the Institutional Review Board of UMDNJ (IRB approval number 0220070186) and the joint Ethics Committee of Peking University Health Sciences Center and Peking University First Hospital (IRB approval number [2007]069). Written informed consent was obtained from all potential subjects before screening for the study. Upon the completion of each experimental session, an honorarium was offered to each subject to compensate them for their time. The questionnaire responses and data files containing subject identifiers were securely stored using either locked filing cabinets or computers

Table 1. Demographic Characteristics of Participants, Measured at the Screening Visits

	Female (n = 62)		Male (n = 63)	
	Mean ± SD	Range	Mean ± SD	Range
Age (yr)	24 ± 1	22–29	24 ± 2	19–33
Height (m)	1.62 ± 0.05	1.52–1.72	1.71 ± 0.06	1.58–1.83
Weight (kg)	53.7 ± 7.2	40.0–75.0	66.3 ± 10.7	51.5–101.0
Body mass index (kg/m^2)	20.6 ± 2.4	16.2–29.3	22.5 ± 2.9	17.8–31.9
SBP (mmHg)	105 ± 8	90–125	116 ± 10	95–138
DBP (mmHg)	68 ± 6	60–85	74 ± 7	60–88

with password protection. By securing the data and ensuring that only the investigators and designated study staff members had access to records, subjects' identities were completely protected in compliance with human subject guidelines.

CLINICAL VISITS

After recruitment and informed consent, each subject completed a medical history, physical examination, routine blood chemistry tests, spirometry, and electrocardiography (ECG) to rule out any medical conditions that would preclude participation. To maximize study personnel efficiency, we collected data from between 10 and 13 subjects on average at a time. Each clinical visit was about 60 minutes in duration and occurred at the same time of day (morning). Subjects were fasting on each test day to reduce extraneous effects on platelet activation markers. Subjects were asked not to use aspirin or nonsteroidal anti-inflammatory drugs for 2 weeks before testing. They were told that acetaminophen was acceptable as a minor analgesic and were given samples for use, if necessary. At each session check-in, subjects were asked if they had used any of the potentially confounding medicines; they were told that a positive answer would not disqualify them, but would only be taken into account in the analysis. Clinical visits proceeded as long as the subject remained otherwise medically qualified, since these medications are known to affect only platelet testing and not any other endpoints used in this study.

Subjects could not have an active upper-respiratory illness (either an infection or allergy) and were rescheduled if they had symptoms in the previous 7 days. Based on the questionnaire data, the physician determined that rescheduling due to upper-respiratory illness was needed for only two subject visits during the entire study (one visit was rescheduled for 2 days later, and the other for 6 days later). The subjects could not have used anti-inflammatory medication for allergies or other respiratory conditions for 2 weeks before a visit. Based on the questionnaire records, none of the subjects had used allergy or anti-inflammatory medications within that time frame before a visit. Indeed, only 6 subjects reported to have had seasonal allergies. Five of these subjects had allergic reactions in either the winter or spring, but not during our study in the summer and early autumn. Only 1 subject reported a history of summer allergies, with the last occurrence 5 years before the study.

Typically, all the subjects worked 8 hours a day, 5 days a week, including some night shifts. For each study subject, visits took place on the same day of the week (as much as possible) and were separated by at least 1 week. To avoid any potential impact from variations in sleeping patterns and unusual activities, visits were scheduled during routine time–activity periods and not after a night shift or travel event (vacations out of town or something similar).

On the day of a clinical visit, subjects reported for a suite of clinical procedures at Peking University First Hospital, under the supervision of the primary field-study physician. There was a quiet room for ECG testing, which was performed on subjects in the supine position. Blood pressure was recorded with sphygmomanometers (blood pressure cuffs) and with stethoscopes superior to the olecranon process on the subjects' arms. Then blood was drawn into heparinized vacuum tubes containing EDTA and aliquoted for measurement of the selected blood markers. To minimize vascular trauma, platelet function specimens were collected using phlebotomy without employing a tourniquet. EBC and eNO samples were collected after the ECG procedure and the drawing of blood. If possible, ECG monitoring was performed first on all subjects, but otherwise the order of the other tests varied among subjects. A urine sample was collected during each clinical visit at a convenient time.

BIOMARKERS AND PHYSIOLOGIC ENDPOINTS

During each clinical visit, physiologic measurements and biologic specimens were collected for each study subject. These were used to measure a large set of biomarkers reflecting specific physiologic functions or biologic pathways, as summarized in Table 2 and described in more detail below.

Exhaled Breath Condensate Collection and pH Measurement

We measured markers of oxidative stress and airway inflammation in collected EBC samples, including pH (hydrogen ions), 8-isoprostane, nitrite, and nitrate. EBC was collected using a Jaeger EcoScreen EBC collector (Erich Jaeger, Germany). The machine was switched on at least 30 minutes before collection to allow the cooling cuff to reach and stabilize at the operating temperature of −20°C. The sealing cap was applied to the cuff to insulate the internal cooling area and to avoid condensation of ambient moisture (which would freeze the lamellar condenser to the cuff when it was inserted). EBC was collected for 20 minutes, during which time subjects were seated, wearing a nose clip, and instructed to breathe tidally. Approximately 2 to 3 mL of condensate were obtained per collection.

After collection, the interface was removed from the cooling cuff, and the sealing cap was then replaced. With

Table 2. Summary of Physiologic Endpoints and Biomarkers Measured

Physiologic Function	Specimen Type	Biomarker/Endpoint	Measurement Principle/Equipment
Pulmonary inflammation and oxidative stress	Exhaled breath condensate	pH	pH meter
		8-Isoprostane	ELISA
		Nitrite and nitrate	HPLC-UV
	Exhaled breath	FeNO	Chemiluminescence analyzer
Autonomic tone/measure of cardiovascular physiology	N/A	HR and HRV	ECG analysis systems
		Blood pressure	Sphygmomanometer
Hemostasis (procoagulation)	Blood	vWF	ELISA
		Platelet aggregation	Photometric aggregometer
		Platelet activation (sCD40L, sCD62P)	ELISA
Systemic inflammation	Blood	Cell counts (WBC, RBC, neutrophils, lymphocytes)	Standard automated clinical methods
		Plasma CRP	ELISA
		Plasma fibrinogen	Immunologic-based chemistry assay
Systemic oxidative stress	Urine	8-OHdG	HPLC-ECD

the condenser held upright, the sample collection vessel was removed, and its contents defrosted. We de-aerated samples with argon gas (350 mL/min for 10 minutes). We then measured EBC pH using an electronic pH meter, which had a resolution of 0.01 units and a working range of pH −2.00 to 16.00 and which was calibrated using standard pH buffer solutions daily before use. We then aliquoted samples in labeled CryoTubes and added an antioxidant mixture (butylated hydroxytoluene, 2 mM in 99% ethanol, 10 µL per mL of sample). Samples were immediately stored at −80°C for later analysis of the target biomarkers. The reusable collection interface was sterilized according to the manufacturer's recommendation. All components were then thoroughly rinsed in double-distilled and de-ionized water to avoid sample contamination. Analyses for specific components of the condensate specimens are described in the following sections.

EBC 8-Isoprostane

We measured concentrations of 8-isoprostane in EBC using a commercially available enzyme-linked immunosorbent assay (ELISA) method (Rapidbio, West Hills, CA, USA). We prepared standards in a supplied phosphate buffer (pH 7.4) containing sodium azide (0.1 g/L), sodium chloride (0.234 g/L), ethylenediaminetetraacetic acid (EDTA) (0.37g/L), and bovine serum albumin (1 g/L). We added sample aliquots (100 µL) to a 96-well plate precoated with rabbit anti-human 8-isoprostane antibodies. The plate was incubated at 37°C for 120 minutes. After removal of the liquid, we added biotin-labeling murine anti-human 8-isoprostane antibody (100 µL) to the plate. After the sample was incubated at 37°C for 60 minutes and washed 3 times with a phosphate buffer solution (pH 7.4, containing 0.05% Tween 20), we added 100 µL of streptavidin/horseradish peroxidase (HRP) to the plate and then incubated at it 37°C for another 60 minutes. The plate was then washed five times with the same phosphate Tween 20 buffer solution and developed with tetramethylbenzidine (TMB) reagent (90 µL per well) for 30 minutes. We stopped the reaction using 2N sulfuric acid (H_2SO_4) (50 µL) and measured the optical density value at 450 nm using a microplate reader. The detection limit for 8-isoprostane was 1.56 pg/mL. This method has been used in previous studies (Montuschi et al. 1999).

EBC Nitrite and Nitrate

We analyzed EBC nitrite and nitrate using a high-performance liquid chromatography (HPLC) system with a UV detector (Waters model 2695 and 2996, respectively; Waters Corp., Milford, MA, USA). The column used for

separation of nitrite and nitrate was IC-Pak Anion HC (4.6 × 150 mm) from Waters. We injected aliquots of EBC samples (20 μL) into the HPLC system running with 10 mM borate/boric buffer at 0.8 mL/min as the mobile phase. The wavelength of the UV detector was set at 214 nm. We prepared external standards using high-purity potassium nitrite and potassium nitrate in deionized water. The detection limits were 7.22 ng/mL for nitrite and 4.43 ng/mL for nitrate. Repeated analyses of samples (n = 8) showed high reproducibility, with a relative standard deviation of 4.0% for nitrite and 2.6% for nitrate.

Fractional Exhaled Nitric Oxide

We measured FeNO using an offline sampling method following the recommendations of the American Thoracic Society/European Respiratory Society (ATS/ERS 2005) and described in an earlier publication (Lin et al. 2011). Subjects were trained in the use of the apparatus before beginning the study. Before sampling, each subject was asked to put the mouthpiece of the device tightly in his or her mouth, inhale deeply, and then exhale to wash the "dead space" from the device. This procedure was repeated twice. All measurements were made with the subjects seated, following 3 to 5 minutes of rest. They inhaled from the functional residual capacity through a mouthpiece with a NO-scrubber attached, thereby inhaling NO-free air, followed by a controlled expiration through the mouthpiece. (Tests confirmed that subjects inhaled ambient air with NO concentration below the detection limit of 0.4 ppb.) A rotameter was used to provide visual guidance to aid subjects and the field technician in maintaining a subject-controlled steady expiratory flow at 150 L/hr, thus improving reproducibility. (The field technician discarded the air sample if the subject's flow rate did not reach the 150 L/hr target.) A resistive pressure of 13 cm H_20 was applied to the exhaled air flow to ensure the closure of the nasopharyngeal vellum, thus preventing contamination by NO from the nose and sinuses. Hence, we collected the exhaled air containing only NO released from epithelial cells into a NO-impermeable aluminum foil bag (Huayuan Gas Center, China). We collected at least 1.5 L of exhaled air, which was more than adequate for the subsequent FeNO analysis using a NO/NO_2/NO_x (nitrogen oxides) chemiluminescence analyzer (model 42i; ThermoScientific, Rockford, IL, USA) within 3 hours of collection. The analyzer had a detection level of 0.40 ppb NO, an accuracy of ± 0.40 ppb, and a detection range of 0 to 100 ppb. The analyzer was calibrated every day using five different concentrations of NO (0–80 ppb) in ultrapure nitrogen (Beijing Haikeyuanchang Practical Gas Co., Ltd., China).

Heart Rate and Heart Rate Variability

We measured HR and HRV using a 12-lead 3-channel MGY-S2 ECG Analysis System (ECG Lab 3.0, Meigaoyi Co., Beijing, China) for 10 minutes per visit. The ECG system software (ECG Lab 3.0, DM Software, Stateline, NV, USA) automatically sorts information into unique morphologic categories (normal, ventricular, paced, artifact, etc.) and then labels each abnormality in a diagnostic strip with its classification, time of occurrence, the corresponding HR, and beat annotations. The software program also allows for the screening and editing of isolated ectopic beats or runs of sustained arrhythmia, and automatically performs data trending in both time and frequency domains. We performed time- and frequency-domain HRV analysis with a fast Fourier transform on the artifact-free and ectopy-free normal R-to-R intervals (the interval between adjacent R-wave peaks indicating the cycle length of a heart beat) according to the currently recommended practice (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). The time-domain parameters we assessed included standard deviation of normal-to-normal (SDNN) R–R intervals and root mean square of successive differences (rMSSD) between adjacent normal cycles. We categorized the frequency spectrum into low-frequency (LF) power (0.04–0.15 Hz) and high-frequency (HF) power (0.15–0.40 Hz). We also computed the ratio of LF to HF. Other HRV parameters assessed using the ECG included very low frequency (VLF) power (0.003–0.04 Hz) and total power (total spectral power of all intervals up to 0.4 Hz).

von Willebrand Factor

An adhesive glycoprotein that is produced by endothelial cells and allows platelets to attach to the subendothelial vessel wall, vWF may serve as an endothelial-derived hemostasis marker of exposure to PM (Pope 1989; Pope et al. 2006). Using 2.7 mL venous blood, we measured vWF protein levels in the plasma using a commercially available ELISA kit (Hushang Biotechnology, Shanghai, China). In this immunologic method using anti-vWF antibodies (rabbit polyclonal), vWF was measured as vWF antigen in subjects' plasma compared with that in a standard species-specific plasma pool (Genc et al. 2011). Hence the vWF levels were reported as a percentage (%).

Platelet Aggregation

We determined with a photometric aggregometer (model LBY-NJ4, Beijing Precil Instrument Co., China) the percentage of total platelets aggregated. We centrifuged fresh blood samples (in 2.7 mL aliquots, within 3 hours of

collection) at 500 to 800 rpm for 10 minutes to obtain platelet-rich plasma (PRP) in the supernatant. An aliquot of the PRP supernatant was then centrifuged at 4000 rpm for 10 minutes to obtain platelet-poor plasma (PPP). We adjusted (standardized) the platelet count in PRP to 200×10^9/L by dilution with PPP. We then incubated 300 µL of the standardized PRP at 37°C and continuously stirred (at 1000 rpm) for 5 minutes before adding a platelet aggregation inducer (epinephrine) to make a final concentration of 4.5 µM PRP. At least 3 minutes after adding the inducer, the percentage of platelets aggregated was determined.

sCD62P and sCD40L

We used soluble sCD62P and sCD40L as biomarkers of platelet activation (Blann et al. 2003; Heeschen et al. 2003; Genc et al. 2011) and measured their concentrations in plasma using commercially available ELISA kits (Rapidbio). For sCD62P, we prepared the standards in a supplied phosphate buffer (pH 7.4) containing sodium azide (0.1 g/L), sodium chloride (0.234 g/L), EDTA (0.37 g/L), and bovine serum albumin (1 g/L). Aliquots of plasma samples (100 µL) were added into a 96-well plate (precoated with rabbit anti-human sCD62P antibodies). The plate was incubated at 37°C for 120 minutes. After removal of the liquid, we added enzyme-labeling murine anti-human sCD62P antibody (100 µL) to the plate. After incubating at 37°C for 60 minutes, the plate was then washed five times with the phosphate buffer solution (pH 7.4, containing 0.05% Tween 20) and developed with o-phenylenediamine reagent (100 µL per well) for 15 minutes. We stopped the reaction with 2N H_2SO_4 (50 µL) and measured the optical density value at 490 nm using a microplate reader. The detection limit of sCD62P in plasma was 1 ng/mL.

For sCD40L, we prepared the standards in a supplied phosphate buffer (pH 7.4) containing sodium azide (0.1 g/L), sodium chloride (0.234 g/L), EDTA (0.37 g/L), and bovine serum albumin (1 g/L). Plasma sample aliquots (100 µL) were added to a 96-well plate (precoated with rabbit anti-human sCD40L antibodies). The plate was incubated at 37°C for 120 minutes. After removal of the liquid, we added biotin-labeling murine anti-human sCD40L antibody (100 µL) to the plate. After the plate was incubated at 37°C for 60 minutes and washed carefully three times with the phosphate buffer solution (pH 7.4, containing 0.05% Tween 20), we added streptavidin/HRP (100 µL) to the plate before incubating at 37°C for 60 minutes. The plate was then washed five times with the phosphate buffer solution (pH 7.4, containing 0.05% Tween 20) and developed with TMB reagent (100 µL per well) for 20 minutes. We stopped the reaction with 2N H_2SO_4 (50 µL) and measured the optical density value at 450 nm using a microplate reader. The detection limit of sCD40L in plasma was 1 ng/mL.

Blood Cell Counts, Plasma Fibrinogen, and C-Reactive Protein

We measured complete blood cell counts and differential leukocyte counts using standard, automated clinical methods in the hematology laboratory of the First Hospital. WBC and RBC counts per unit volume were reported and analyzed in this study, as these blood indices may reflect systemic inflammation associated with PM exposure (Seaton et al. 1999; Symons et al. 2006).

Plasma fibrinogen and CRP are acute phase proteins that are produced by inflammatory mediators acting on the liver, and thus may serve as biomarkers of systemic inflammation (van Eeden et al. 2001; van Eeden and Hogg 2002; Shishehbor et al. 2003). Both biomarkers were analyzed in the local hospital's hematology laboratory using standard procedures. Plasma fibrinogen was analyzed within 4 hours of venous blood collection using an automated analyzer, ACL9000 (Beckman Coulter Co., Beijing Branch, China). CRP concentrations were measured in EDTA (anticoagulant)–treated plasma using a clinical immunonephelometric assay with an automated nephelometer. The detection limit for CRP was 0.3 mg/mL. However, because of the low accuracy of the actual CRP values reported, these values were used cautiously in the statistical analysis and interpretation of results.

Urinary 8-Hydroxy-2′-Deoxyguanosine

We diluted an aliquot of each urine sample (1.5 mL) with 1.5 mL of potassium dihydrogen phosphate buffer (0.1 M, pH 6) and then forced it through a solid phase extraction cartridge previously conditioned with 5 mL methanol, 5 mL deionized water, and 5 mL potassium dihydrogen phosphate buffer (0.1 M, pH 6). After washing the cartridge with 3 mL deionized water and 1.5 mL of the same buffer solution, we dried it with a vacuum system for 10 minutes and then eluted it with a 2 mL solution of 30% methanol in deionized water. We analyzed the eluted solution (with a sample injection volume of 20 µL) using a HPLC system coupled with an electrochemical detection (ECD) system under the following conditions. We used a µBondapak C18 analytical column (Waters Corp.). The mobile phase program involved the use of two solutions: Solution A consisted of 93.5% deionized water and 6.5% methanol with citric acid (2.4 g/L), sodium acetate (2.05 g/L), acetic acid (0.6 g/L), sodium hydrate (1.2 g/L),

and EDTA (20 mg/L); and Solution B consisted of 90% deionized water and 10% methanol with citric acid (2.4 g/L), sodium acetate (2.05 g/L), acetic acid (0.6 g/L), sodium hydrate (1.2 g/L), and EDTA (20 mg/L). The mobile phase was run under a gradient program at a 1 mL/min flow rate. It consisted of 100% Solution A for the first 12 minutes, switched linearly (after 2 minutes) to 100% B, which was maintained for 17 minutes, and then switched (after 1 minute) to 100% A, which was maintained for 8 minutes until the end (total run time was 40 minutes). We used an ECD detector (model WA2465, Waters Corp.) with the electrode potential set at +0.6 V, the range at 50 nA, and the time constant at 1.0 second (De Martinis and Bianchi 2002). The method detection limit was 0.5 ng/mL. Concentrations of urinary 8-OHdG, normalized by urinary creatinine concentrations, were reported in milligrams per mole.

MEASUREMENTS OF AIR POLLUTANTS AND WEATHER PARAMETERS

We conducted a comprehensive characterization of air pollution, covering the entire time window of the panel study (June 2, 2008, to October 30, 2008; see Figure 3). All the air samplers and monitors were collocated at a secured spot (the rooftop of a seven-story building located in the center of the hospital campus). All the real-time monitors were operated continuously throughout the entire measurement period (from June 2 to October 30). $PM_{2.5}$ mass and constituent measurements were made on a 24-hour basis, starting from approximately 10 AM. The pollutant species and their measurement time resolutions are summarized in Table 3. The span of time used to calculate the daily average for all the pollutants and the weather parameters was from 10 AM to the time of the last data point before 10 AM on the next day.

We selected the $PM_{2.5}$ constituents based on the following rationale: SO_4^{2-} is a major component of $PM_{2.5}$ mass and is formed through photochemistry-driven oxidation of SO_2 in the atmosphere; hence SO_4^{2-} is often regarded as an indicator of regional sources of fossil fuel combustion. In contrast, EC reflects local emissions especially from the combustion of diesel fuel and/or coal. Given this, we chose EC and SO_4^{2-} as crude indicators of local (predominantly traffic) and regional sources, respectively. Both local emissions and regional transport contribute to OC levels. In addition, EC, OC, and SO_4^{2-} have distinct chemical properties (e.g., solubility and surface reactivity), implying potential differences in their toxicity (Siegel et al. 2004; Zielinska et al. 2010).

Particle Number Concentrations

Our plan was to measure the number concentrations of particles with various size ranges using a scanning

Table 3. Summary of Pollutant Species, Measurement Time Resolution, and Measurement Techniques

	Time Resolution	Sampling Equipment / Measurement Principle or Equipment
Particulate Matter		
Particle number (13–764.7 nm)	Continuous (10 min)	TDMPS
$PM_{2.5}$ mass	24 hr	Cyclone with Teflon filter / gravimetry
$PM_{2.5}$ mass	Continuous (1 hr)	TEOM
EC/OC in $PM_{2.5}$	24 hr	Cyclone with quartz-fiber filter / thermal-optical EC/OC analyzer
PAHs in $PM_{2.5}$	24 hr	Cyclone with quartz-fiber filter / GC–MS
Ions in $PM_{2.5}$	24 hr	Cyclone with Teflon filter / IC
Elements in $PM_{2.5}$	24 hr	Cyclone with Teflon filter / ICP–MS
Gases		
O_3	Continuous (1 hr)	UV spectrometer
NO, NO_2, NO_x	Continuous (1 hr)	Chemiluminescence analyzer
CO	Continuous (1 hr)	Non-dispersive infrared detector
SO_2	Continuous (1 hr)	Fluorescence detector
Meteorologic Parameters		
Temperature	Continuous (1 hr)	Met One meteorology system
RH	Continuous (1 hr)	

mobility particle sizer (SMPS) (TSI model 3080, TSI Inc., St. Paul, MN, USA), which was collocated with other air samplers and monitors at the HEART study site (Peking University First Hospital). The SMPS was assembled with a long differential mobility analyzer (TSI model 3081) and a condensational particle sizer (TSI model 3025A) to measure particle number size distributions from 14.1 nm to 736.5 nm with a time resolution of 5 minutes.

Because of technical problems with the SMPS system, we failed to measure particle number concentrations for the pre- and during-Olympics periods. However, at the main campus of Peking University, about 7 km from the hospital, there was a twin differential mobility particle sizer (TDMPS), consisting of two Hauke-type differential mobility analyzers (TSI model 3010) and two condensation particle counters (TSI model 3025), which functioned normally and collected data covering the pre-, during, and post-Olympics periods as defined in this study. The TDMPS measured particle number concentrations in 26 size bins (ranges) between 13 nm and 764.7 nm at 10-minute intervals. Hence we were able to use particle number concentrations measured at this site in our data analysis. We analyzed size-resolved concentration data to determine particle size distributions by period. Because of time and resource constraints, we analyzed only TPN in relation to biomarkers in this report.

On 27 days during the post-Olympics period, both the SMPS system located at the hospital and the TDMPS system located on the main campus worked normally, allowing us to compare the concentrations of TPN measured in the same 24-hour periods at the two sites. As shown in Figure 4, the linear regression coefficient (r^2) was 0.6963; and the concentrations measured at the hospital grounds appeared to be systematically lower than those measured at the main campus. Because the total particle size range (14.1 nm–736.5 nm) measured with the SMPS system at the First Hospital was slightly smaller than the total particle size range (13 nm–764.7 nm) measured by the TDMPS at the main campus, it was reasonable to expect that the SMPS would measure lower concentrations. However, the slope of the regression line was 0.5022, meaning that the SMPS concentrations were about half the TDMPS concentrations. This large difference may be due to other reasons, including the inherent difference between the two systems and/or actual spatial differences between the two sites. Because of practical limitations, we did not compare the two systems side by side to assess any inherent difference between them. Nonetheless, it is still useful to use TPN concentrations measured at the main campus site given the relatively high correlation between the measurements made at the two sites.

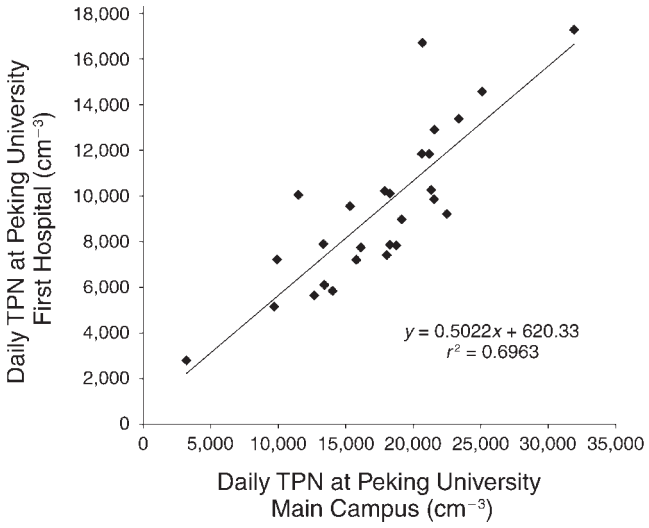

Figure 4. Concentrations of TPN measured at the Peking University First Hospital site (by SMPS; 14.1–736.5 nm) vs. those measured at the Peking University main campus site (by TDMPS; 13–764.7 nm). All data points (n = 27) were 24-hour means, averaging from 10 AM to 10 AM the next day.

$PM_{2.5}$ Mass Concentration

We used two techniques to determine $PM_{2.5}$ concentrations: gravimetric analysis and the tapered-element oscillating microbalance (TEOM) method.

Gravimetric Measurement We collected $PM_{2.5}$ on Teflon filters using a Quad Channel Ambient Particulate Sampler equipped with a 2.5 µm impactor (TH-16A, Tianhong Inc., China) and operating at a flow rate of 16.7 L/min. We weighed the Teflon filters before and after the sampling using an analytical balance (Mettler Toledo AX105DR) with a sensitivity of 10 µg after preconditioning for 24 hours at constant humidity (RH = 40% ± 3%) and temperature (20° ± 1°C). We determined $PM_{2.5}$ mass concentrations by dividing PM mass collected on the filter by the total sampling volume.

TEOM Method We used a TEOM 1400a Ambient Particulate Monitor (Thermo Electron Corp., NY, USA) to measure $PM_{2.5}$ real-time mass concentrations. The TEOM method has been designated by the U.S. Environmental Protection Agency (EPA) as equivalent to the gravimetric method (EPA Designation No. EQPM-1090-079). This is an inertial measurement technique that operates by measuring changes in the resonant frequency of an oscillating element as a function of increases in particle mass collected on a filter attached to the element. Changes in the element's resonant frequency are sampled electronically in quasi real time, providing both continuous and time-averaged

measures of mass accumulation that are directly proportional to instantaneously measured and time-averaged $PM_{2.5}$ mass concentrations in the air, respectively. We collected both hourly and 24-hour averages of $PM_{2.5}$ concentrations using the TEOM method with the sampling inlet temperature set at 50°C.

Comparison Between TEOM and Gravimetric $PM_{2.5}$ Data We ran a linear regression analysis to compare $PM_{2.5}$ mass concentrations measured by the two methods. As shown in Figure 5, the two methods agreed highly with an r^2 of 0.9387 and a near-unity slope. However, the TEOM method appears to underestimate $PM_{2.5}$ mass concentration by approximately 11 μg/m^3 (intercept = 11.097 μg/m^3). In this report, we used the gravimetrically derived data as the primary data for all analyses, except for the few dates (n = 6) (before the start of visit 3 in the during-Olympics period) when gravimetric data were missing; to derive data for those dates, we used the equation shown in Figure 5 to normalize TEOM data to make them comparable to the gravimetric data.

Species and Total $PM_{2.5}$ Mass We also assessed the relative contributions of the species to the total $PM_{2.5}$ mass. To do that, we grouped the measured species into the following categories: (1) organic matter (OM), defined as 1.4 times the OC concentration, based on previous studies of PM in Beijing (Zheng et al. 2005); (2) *EC*; (3) SO_4^{2-}; (4) NO_3^-; (5) NH_4^+; (6) other ions (sum of Na^+, K^+, Mg^{2+}, Ca^{2+}, F^-, and Cl^-); (7) PAHs (sum of the 14 individual PAHs); (8) transition metals (sum of the following 13 elements: Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Cd, Th, and U); and (9) other unknown species representing the difference between $PM_{2.5}$ mass and the sum of all the species specified in the eight categories.

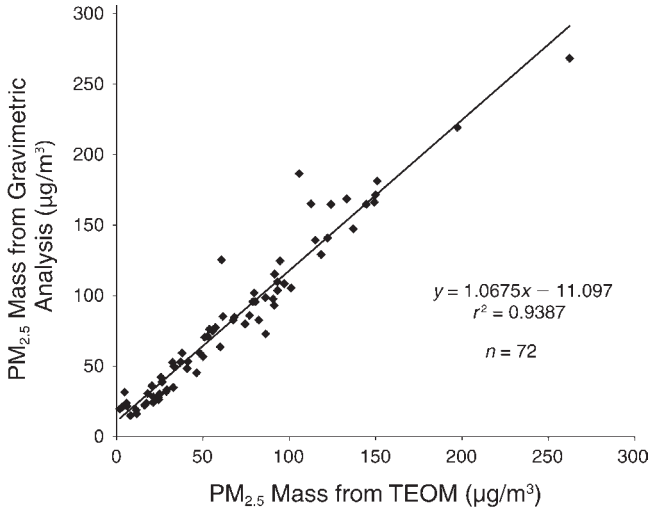

Figure 5. $PM_{2.5}$ concentrations measured using the gravimetric method vs. those measured using the TEOM method. The two types of measurements were made at the same site and the same time. All data points were 24-hour means, averaging from 10 AM to 10 AM the next day.

Elemental Carbon and Organic Carbon

We precleaned quartz-fiber filters to remove carbonaceous contaminants by firing them at 500°C for 5.5 hours and then stored them in petri dishes in the freezer at −4°C until use. After $PM_{2.5}$ was collected, we punched out a 1.45 cm × 1 cm section from each quartz filter and used it to determine OC and EC with an OC/EC analyzer (Sunset Laboratory, Tigard, OR, USA) following the standard protocol of the National Institute for Occupational Safety and Health Reference Method #5040 (Birch 1999).

Polycyclic Aromatic Hydrocarbons

We collected particle-phase polycyclic aromatic hydrocarbons (PAHs) on quartz-fiber filters at a sampling flow rate of 16.7 L/min. PAHs collected on filters were extracted by dichloromethane using a Dionex ASE300 Extractor (Dionex, Sunnyvale, CA, USA). Resulting extracts were concentrated to 1 mL using a gentle nitrogen flow through the extract solution. Aliquots of the final extracts were analyzed for PAHs employing a gas chromatography–mass spectroscopy (GC–MS) system (Agilent GC model 6890 and MS model 5973N, Agilent Technologies, Inc., Santa Clara, CA, USA). Both external and internal standards (National Institute of Standards and Technology [U.S.]) were used to quantify the following 15 PAHs: naphthalene, acenaphthylene, fluorine, phenanthrene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*e*]pyrene, benzo[*a*]-pyrene, indeno[1,2,3-*cd*]pyrene, dibenzo[*a,h*]anthracene, and benzo[*g,h,i*]perylene. (Because we had some internal standard problems with naphthalene, along with the well-recognized difficulty [Gundel et al. 1995] in measuring this relatively volatile compound in the particulate phase, we removed it from our PAH list.) The column used for separation was a 30 m × 0.25 mm inner-diameter-fused silica capillary column, coated with a cross-linked phenyl methyl silicone. The carrier gas was helium with a constant flow rate of 1.20 mL/min. The initial oven temperature was 40°C for the first 2 minutes, and then was increased at a ramping rate of 10°C/min to the final temperature of 300°C, which was maintained for 10 minutes. The mass spectrum operation conditions were as follows: source temperature, 250°C; gas chromatography interface temperature, 300°C; the emission current, 350 μA; the electron energy, 70.0 V (nominal); and the detector voltage, 350.0 V (He et al. 2006; Huang et al. 2006).

Inorganic Ions

We designated filters used in one of the four channels in the $PM_{2.5}$ sampler for analysis of the following nine water-soluble anions and cations: fluoride (F^-), chloride (Cl^-), nitrate (NO_3^-), SO_4^{2-}, ammonium (NH_4^+), calcium (Ca^{2+}), sodium (Na^+), magnesium (Mg^{2+}), and potassium (K^+). Samples were extracted using 10 mL deionized water in an ultrasonic bath for 30 minutes at room temperature, and the analysis was performed using ion chromatography (IC) (Dionex ICS-2500, Dionex). We used an AS11 column (4 mm) with an AG11-HC (4 × 50 mm) guard column and an anion trap column (ATC-3, 9 × 24 mm, for 4 mm) for anion detection with an eluant of 0.4 to 6 mM/L sodium hydroxide (1.2 mL/min, gradient). We analyzed cations using a CG-12A (4 × 50 mm) guard column and CSRS-I suppressor. The eluant was 20 mM/L methylsulfonic acid with a flow rate of 1.0 mL/min. Field and laboratory blanks were analyzed using the same method, and the concentrations on these controls were all below the detection limits. More detailed information about the method can be found in previous papers (Hu et al. 2005; Guo et al. 2010).

Elements

We used Teflon filters installed in another channel of the $PM_{2.5}$ sampler for analysis of the following 24 trace elements: sodium (Na), magnesium (Mg), aluminum (Al), phosphorus (P), potassium (K), calcium (Ca), titanium (Ti), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), molybdenum (Mo), cadmium (Cd), barium (Ba), thallium (Tl), lead (Pb), thorium (Th), and uranium (U). This analysis was done in a commercial laboratory using a common inductively coupled plasma mass spectrometer facility with a plasma forward power of 1350 W (Agilent 7500C ICP–MS). Instrument calibration was achieved using multi-element standards made up in a 5% nitric acid solution prepared by Agilent Technologies (part # 518324682).

Gaseous Pollutants and Weather Parameters

We measured gaseous pollutants (O_3, CO, SO_2, NO, NO_2, and NO_x) using instruments from Ecotech Pty. Ltd. (Knoxfield, VIC, Australia), including an EC9810B O_3 analyzer with a detection limit of 0.5 ppb, an EC9830 CO analyzer with a detection limit of 0.5 ppb, an EC9841B $NO/NO_2/No_x$ analyzer with a detection limit of 0.4 ppb, and an EC9850B SO_2 analyzer with a detection limit of 0.3 ppb. These automated monitors were maintained and calibrated following the manufacturer's protocols. We monitored ambient temperature and RH at the same site using a Met One meteorology system (Grants Pass, OR, USA). We extracted precipitation data from a publicly available Web site that reported rainfall for Beijing (Weather Underground, *www.wunderground.com/weatherstation/WXDailyHistory.asp?ID=IBEIJING13*).

We obtained hourly measurements for all these gaseous species using real-time monitors (see Table 3). To maintain consistency with filter-based $PM_{2.5}$ measurements, we computed daily means for these species using the same start time (~10:00 AM) and end time (the last data point before 10 AM) in each 24-hour period. Because O_3 had particularly large diurnal variations, we also computed maximum 1-hour average concentration within a 24-hour period (referred to as "O_3 max").

STATISTICAL METHODS AND DATA ANALYSIS

Descriptive Statistics

We performed descriptive statistics for each air pollutant measured and for each health endpoint (biomarker). We calculated means, standard deviations, and quartiles, as well as minimums and maximums, for each visit and/or each period (i.e., pre-, during-, and post-Olympics periods), as described below. We also calculated the proportion above the minimum detectable level (data not shown). When calculating means and standard deviations, values that were below the detectable limit were set to half of the detectable limit.

All biomarkers, except CRP and 8-isoprostane, were evaluated as continuous responses using linear modeling techniques, as described below. CRP and 8-isoprostane concentrations were dichotomized before statistical analysis because of the large number of nondetectable values as well as a skewed distribution of the remaining values. Further, they were evaluated using hierarchical logistic regression models, also described below.

Comparison of Pollutant Concentrations by Period

We used graphs and box plots illustrating descriptive statistics for pollutant levels and meteorologic variables to examine the distributions and correlations of pollutant levels across periods. To compare air pollutant concentrations in the pre-, during-, and post-Olympics periods, we employed time-series regression models to assess the between-period differences for significance. We used the following model structure:

$$Y_t = \alpha_0 + \alpha_1[I(t \in \text{During-Olympic Period})] + \alpha_2[I(t \in \text{Post-Olympic Period})] + \varepsilon_t$$

where t represents the date of measurement, Y_t denotes the pollutant concentration at date t, α_0 denotes the mean for the pre-Olympics period, α_1 denotes the mean difference between the during- and pre-Olympics concentrations, α_2 denotes the mean difference between the post- and pre-Olympics concentrations, and ε_t denotes random error, which was modeled via an auto-regressive and moving average of order 1. This autoregressive moving-average model (1,1) structure was determined by the autocorrelation function (ACF) plot, partial ACF plot, and Akaike Information Criterion (AIC) values. (The term *I(x)* is an indicator function with a value of 1 if condition *x* holds, and a value of 0 if otherwise.) Linear contrasts were made to compare between-period differences for each pollutant. We conducted the time-series regression analyses using the function "gls" from the R software nlme package. The "gls" function allowed gaps between periods. We compared the unadjusted period-specific means of air pollutant concentrations estimated by the autoregressive moving-average models with the observed period-specific averages (sample means) and found that they were close. We also used the Durbin–Watson statistic to examine the residual auto-correlations and found that it was small (<2), indicating minimal residual auto-correlations.

Analyses of Biomarkers with Continuous Values

We used mixed-model analyses to examine period (pre-, during-, and post-Olympics) effects as well as, separately, associations between pollutant and biomarker levels, controlling for temperature, RH, sex, and day of the week. In order to account for correlation within subjects, we compared alternative correlation structures using AIC. The model that was consistently chosen as the best across biomarkers was the simplest model in which a random effect for subject induced equicorrelation between all observations within subject. Next, we adjusted for temperature and RH using natural splines with the degrees of freedom chosen to minimize AIC; same-day temperature and RH were included in each model. We also accounted for additional seasonal differences by including the cumulative averages of temperature and RH of up to 7 days, using natural splines if these terms resulted in lower AICs. Because partial regression plots suggested that allowing more than 3 degrees of freedom (df) resulted in overfitting, we allowed only up to 3 df for all splines. We used the final models to examine period effects and pollutant–biomarker associations as follows.

Comparison of Biomarker Levels by Period Our study design has a unique quasi-experimental "A-B-A" structure enabling the comparison of biomarker changes by period (pre-, during-, and post-Olympics), as distinct from previous studies that have assessed biomarker change as a function of day-to-day fluctuations in ambient pollutant levels (Adamkiewicz et al. 2004; Chuang et al. 2007; Barraza-Villarreal et al. 2008; Delfino et al. 2009). Both the adjusted and unadjusted effects of the period (pre-, during- and post-Olympics) were examined by adding indicator variables for period into the random effects model with and without the adjustment for temperature, RH, and day of the week (as discussed above). We used contrasts and related F-tests to examine the pairwise comparisons between periods.

Relationships Between Pollutants and Biomarkers We evaluated the relationship between a biomarker and a pollutant across the entire study period (including the pre-, during-, and post-Olympics periods). Pollutant concentrations were measured 1 to 7 days before biomarker measurements were taken (referred to as lag day 0 to lag day 6). We examined the associations, while controlling for temperature, RH, and day of the week for biomarker measurement, by adding the pollutant concentrations to the mixed linear models described above (but without period indicators). Specifically, analyses examined adjusted associations between average biomarker levels and pollutant concentrations averaged over the last 24 hours (lag 0), 24 to 48 hours (lag 1), 48 to 72 hours (lag 2), and so on, up to a 7-day lag (lag 6). For all pollutant–biomarker combinations, we created "lag plots" representing the change in biomarker level associated with one interquartile range (IQR) increase in pollutant for lags 0 through 6. These lag plots were a series of single-lag models. Because some pollutants were correlated with others, we used two-pollutant models to examine whether the pollutant–biomarker associations from the single-pollutant analyses were consistent with the effects of that pollutant when controlling for another pollutant. These double-pollutant models included only one lag for each pollutant — the lag demonstrating the strongest statistical significance.

Measurements of EBC nitrite, EBC nitrate, FeNO, and all the HRV variables were log-transformed before being analyzed in the linear models, because the values of these biomarkers were right-skewed. In all analyses, biomarker outliers (identified as those at least 3 standard deviations away from the mean) were eliminated from the modeling. The identification of outliers was done after the biomarker values were log-transformed. Only a very small number of data points were removed for only a few biomarkers. For example, out of a total of 748 observations, 1 RBC value (0.1%) and 9 fibrinogen values (1.2%) were identified as outliers. None of the log-transformed biomarkers had outliers.

Dichotomized Analyses

The distributions of CRP and EBC 8-isoprostane concentrations were highly skewed with a large percentage of nondetectable values. Hence, analyses for these biomarkers should be considered exploratory and differ somewhat from those described above. Specifically, we dichotomized the biomarker levels based on data distribution into detectable or nondetectable categories, or into the first 75th percentile of the entire data set and the last quarter.

We tested period and pollutant effects for these dichotomized biomarkers using hierarchical logistic regression (with random effects for subject), controlling for temperature and RH via natural splines, as described above, for the mixed linear models as well as sex and day of the week. In the period comparison (e.g., pre-Olympics period vs. during-Olympics period), we assessed the fraction of the samples above the detection limit for both biomarkers. However, we assessed pollutant–biomarker associations only for EBC 8-isoprostane, because the low "clinic-grade" quality of the CRP data meant they were not useful for any further exploration in sophisticated models. We created lag plots to display the percent change in odds ratios for getting a "higher" (above the 75th percentile) value of 8-isoprostane associated with one IQR increase in pollutant level.

Additional details of the statistical models and sample programming codes are provided in Appendix G.

Sensitivity Analyses

We conducted sensitivity analyses to evaluate the robustness of our models to the adjustments for meteorologic parameters (temperature, RH, and rainfall). In one set of sensitivity analyses, we removed temperature and/or RH if they were not statistically significant predictors of a change in biomarker level, as well as their moving averages, from multivariate analyses examining the effect of single pollutants on the biomarkers. We plotted the effect estimates from these analyses and compared them with the results from the models including both variables. Next, when using the single-pollutant models, we excluded observations made on days that had greater than 1 mm of precipitation. Finally, while the pre- and during-Olympics periods were during the summer, the post-Olympics period occurred during the autumn, which could have led to some differences in biomarker levels as well as possible residual confounding by unmeasured factors, other than meteorologic factors, that varied across periods. Although subjects whose lifestyles remained relatively stable across the time periods were deliberately chosen, many factors may have varied between periods. For example, there may have been different levels of infections and allergens in circulation or changes in overall behavior, such as time spent outdoors, between periods. While the inclusion of the moving averages of temperature and RH was meant to capture some of this variation, it is possible that we did not catch all of the residual confounding by season. Thus, we conducted two additional sets of analyses to further account for possible confounding by season or period. First, we reanalyzed the pollutant–biomarker relationships while excluding all post-Olympics period observations. Second, we studied within-period effects of the single pollutants by including period indicators as covariates. These sets of analyses were conducted for several biomarkers that showed statistically significant and consistent effects in the main (original) analyses.

We also conducted sensitivity analyses on the two-pollutant models, which were designed to assess the robustness of the estimates associating individual pollutants with the biomarkers. As described above, in the main analysis, we chose the lags with the lowest *P* values for each pollutant to include in the double-pollutant analysis in order to maximize the amount of variation in the biomarkers that would be accounted for by the added secondary pollutant. However, because pollutant levels on the same day tended to be more correlated and hence might pose more of a confounding problem, we conducted copollutant models in which the lag for the second pollutant was the same as the selected lag for the main pollutant of interest.

Finally, the use of random rather than fixed subject effects (or conditioning on the subject's overall mean outcome) is debatable, because it requires drawing some information from between (rather than only within) subjects, which is open to confounding by individual time-invariant risk factors. For this reason, we conducted another set of sensitivity analyses for selected biomarkers in which we included fixed rather than random subject effects.

RESULTS

CHARACTERISTICS OF AIR POLLUTION

Concentrations of Air Pollutants Before, During, and After the Olympics

We measured concentrations of $PM_{2.5}$ and a large number of $PM_{2.5}$ chemical constituents (as shown in Tables A.1–A.3). Because of time and resource constraints, we

focused on selected pollutants in our analysis of relations between biomarkers and pollutants.

Concentrations for these selected pollutants are summarized in Table 4. The table includes results for $PM_{2.5}$ mass, SO_4^{2-}, EC, and OC, as well as results for TPN (i.e., number concentrations of all particles in the size range between 13 nm–764.7 nm) and the gaseous pollutants SO_2, CO, NO_2, and O_3.

Mean and median concentrations of $PM_{2.5}$ mass and constituents in the during-Olympics period decreased markedly from their respective pre-Olympics levels (see Table 4 for mean concentrations and Table P.1 in Appendix P for median concentrations using raw data). Similar findings were observed for the gaseous pollutants except for O_3 (see Tables 4 and P.2). Both the 24-hour average and the 1-hour maximum for O_3 had higher mean and median concentrations in the during-Olympics period than in the pre-Olympics period. The mean concentrations were associated with large standard deviations (SDs) and IQRs even within a single period (data not shown), indicating large day-to-day variations both within each period and across the three periods.

Using a time-series regression approach described in the previous section, "Statistical Methods and Data Analysis," we computed the means and 95% confidence intervals for between-period percent changes in pollutant concentrations, plotted in Figure 6. As shown in Figure 6A, mean concentrations of the following pollutants were significantly reduced in the during-Olympics period compared with those in the pre-Olympics period: EC, 36% reduction; TPN, 22% reduction; SO_2, 60% reduction; CO, 48% reduction; and NO_2, 43% reduction. Mean $PM_{2.5}$ concentration was reduced by 27%, but the upper limit of the 95% CI was a 9% increase. Two constituents of $PM_{2.5}$ — SO_4^{2-} and OC — each had a reduction in mean concentration but a wide 95% CI crossing zero. Both 24-hour average and 1-hour maximum concentrations of O_3 increased from pre- to during-Olympics periods, as did ambient temperature (data not shown).

The post-Olympics period encompassed early autumn in Beijing; thus mean temperature for this period (16.8°C) was lower than that for the pre-Olympics period (25.1°C) and the during-Olympics period (27.7°C), and mean RH was lower for the post-Olympics period (48.6%) compared with the pre-Olympics period (66.6%) and the during-Olympics period (64.8%) (data not shown). In the post-Olympics period, we observed increases in mean concentrations for all the pollutants except SO_4^{2-} and O_3 (Figure 6B) relative to the during-Olympics mean concentrations, although the changes were not statistically significant for $PM_{2.5}$, SO_2, and CO.

There were no significant differences between post-Olympics and pre-Olympics mean concentrations for $PM_{2.5}$ and SO_2. Mean concentrations of SO_4^{2-}, CO, and O_3 were lower in the post-Olympics period, whereas mean concentrations of EC, OC, TPN, and NO_2 were higher in the post-Olympics period (Figure 6C).

Table 4. Air Pollutant Statistics by Period Based on Time-Series Model[a]

	Pre-Olympics		During Olympics		Post-Olympics		Period Difference	
Air Pollutants	*n*	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE	During − Pre Mean (95%CI)	Post − During Mean (95%CI)
SO_2 (ppb)	35	7.45 ± 1.17	24	2.97 ± 1.33	32	6.81 ± 1.22	−4.48 (−7.94 to −1.02)[b]	3.84 (0.31 to 7.37)[b]
NO_2 (ppb)	35	25.60 ± 3.66	33	14.61 ± 3.76	32	41.39 ± 3.81	−10.99 (−21.26 to −0.71)[b]	26.78 (16.29 to 37.26)[c]
O_3 (ppb)	35	31.84 ± 3.75	33	39.60 ± 3.85	32	15.12 ± 3.91	7.75 (−2.78 to 18.29)	−24.48 (−35.24 to −13.72)[c]
O_3 max (ppb)	35	66.47 ± 7.10	33	80.23 ± 7.30	32	42.20 ± 7.41	13.76 (−6.19 to 33.71)	−38.03 (−58.42 to −17.65)[c]
CO (ppm)	35	1.23 ± 0.13	33	0.64 ± 0.14	32	0.81 ± 0.14	−0.59 (−0.97 to −0.22)[b]	0.17 (−0.21 to 0.56)
$PM_{2.5}$ (μg/m^3)	35	98.9 ± 14.7	33	71.9 ± 15.1	32	85.3 ± 15.3	−27.0 (−68.3 to 14.3)	13.3 (−28.8 to 55.5)
EC (μg/m^3)	35	2.2 ± 0.3	28	1.4 ± 0.3	31	3.4 ± 0.3	−0.80 (−1.7 to 0.1)	1.9 (1.0 to 2.8)[c]
OC (μg/m^3)	35	8.8 ± 1.6	28	6.8 ± 1.7	31	15.0 ± 1.7	−1.97 (−6.6 to 2.6)	8.2 (3.5 to 12.9)[c]
SO_4^{2-} (μg/m^3)	35	26.5 ± 5.8	28	23.0 ± 6.4	29	13.7 ± 6.2	−3.5 (−20.4 to 13.5)	−9.3 (−26.7 to 8.1)
TPN (/m^3)	35	16,480 ± 1276	30	12,853 ± 1389	30	19,477 ± 1367	−3627 (−7323 to 70)	6624 (2804 to 10443)

[a] TPN indicates total particle number ranging from 13 nm to 764.7 nm; O_3 max is the maximum 1-hr average concentration within a 24-hr period. All samples were above the detection limit.

[b] $P < 0.05$.

[c] $P < 0.01$.

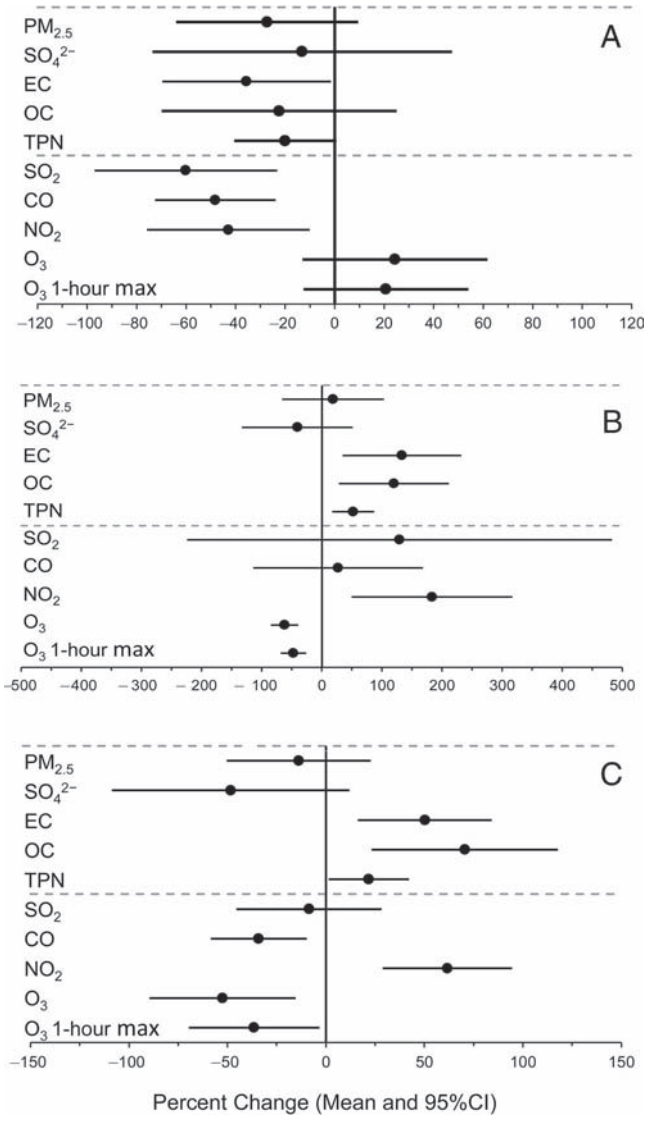

Figure 6. Estimated means and 95% confidence intervals for percent changes in air pollution levels. A: from the pre-Olympics to the during-Olympics period; **B:** from the during-Olympics to the post-Olympics period; and **C:** from the pre-Olympics to the post-Olympics period. The estimates were based on time-series regression models.

Particle Size Distribution

Using 26 bins spanning from 13 nm to 764.7 nm in size, the TDMPS measured particle number concentrations, which can be used to determine particle number size distribution. We used the common plotting technique to present particle size distributions, specifically, comparing log-transformed number concentrations normalized by particle diameter to particle diameters (on a log scale) within a specific size bin. As shown in Figure 7, we compared particle size distributions by period (pre-, during-, and post-Olympics) using the period means based on daily concentrations of particles in each of the 26 size bins. We observed the following: (1) the during-Olympics period had the lowest mean particle concentrations in all size bins; (2) the post-Olympics period had the highest number concentration for particles with a diameter <56 nm compared with the other two periods; (3) differences in particle mean concentration between the pre- and the during-Olympics period were largest when the diameters were in the range of 108 nm to 127 nm; (4) the highest particle number concentrations were for particles in the 66.4–78.1 nm size bins in the pre-Olympics period, 47.9–56.4 nm bins in the during-Olympics period, and 29.4–34.6 nm bins in the post-Olympics period; and (5) overall, as expected, larger-size particles (closer to 764.7 nm) had substantially lower number concentrations.

Because particle mass concentrations have been commonly used in previous PM health effects studies and are currently used in health-based regulatory standards, it is useful to understand particle size distribution by mass (in addition to distribution by number). For this reason, we estimated the mass median particle diameter for each of the three periods, by calculating volume concentrations with the assumption that all particles in a size bin were spheres with the diameter equal to the lower end of the size bin range. Then we obtained mass concentrations by assuming all particles had a density of 1 g/cm^3. We then plotted the cumulative percentage of mass concentrations as a function of particle diameter (using the lower end of each size bin, on a log scale) (see Figure 8). Based on these plots derived from period-specific means, the estimated mass median particle diameters were 423 nm for the pre-Olympics period, 363 nm for the during-Olympics period, and 381 nm for the post-Olympics period.

$PM_{2.5}$ Composition

We measured the following $PM_{2.5}$ species: 9 water-soluble ions (NH_4^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, SO_4^{2-}, NO_3^-, F^-, and Cl^-), 14 PAHs (acenaphthylene, fluorine, phenanthrene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*e*]pyrene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenzo[*a*,*h*]anthracene, and benzo[*ghi*]perylene), and 24 elements (Na, Mg, Al, P, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Ba, Tl, Pb, Th, and U). Concentrations of these species (mass per cubic meter of air) are summarized by period in Appendix A.

Fractional contributions (%) of these species to total $PM_{2.5}$ mass are presented by period in Figure 9. The OM fraction in $PM_{2.5}$ increased from 13.1% in the pre-Olympics period to 17.3% in the during-Olympics period

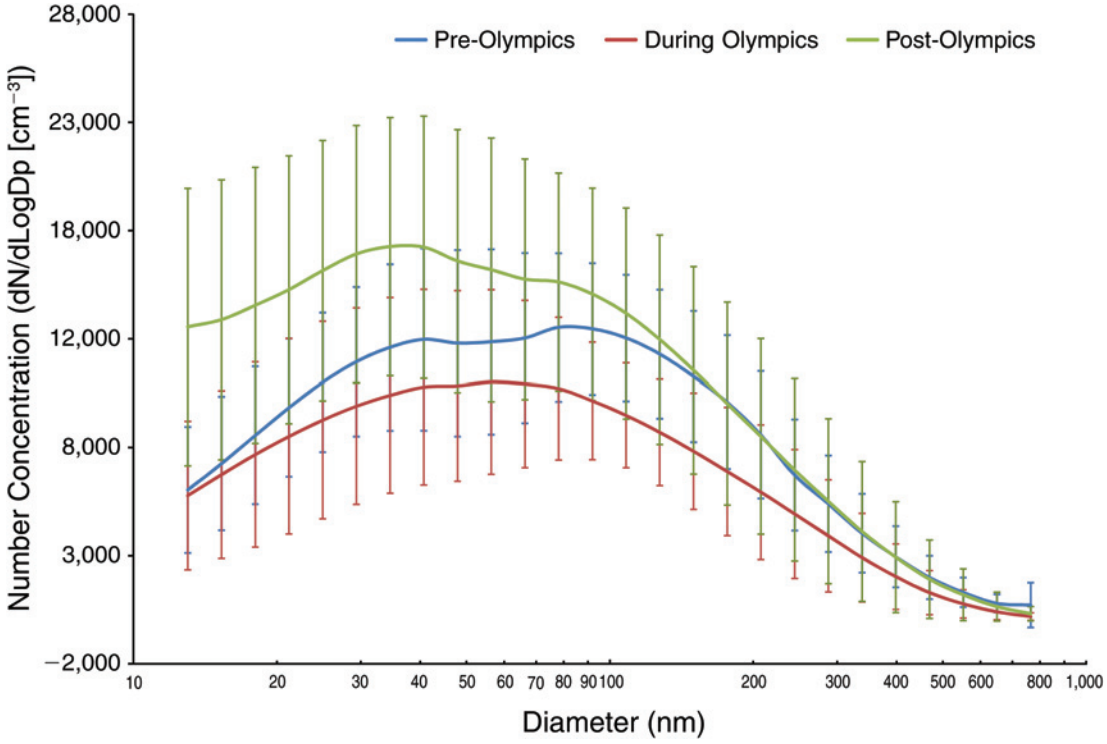

Figure 7. Number-based particle size distribution by period, showing means and standard deviation bars, based on 24-hour average data (10 AM to ~10 AM next day). Particle number concentrations were measured using a TDMPS system with 26 size bins within the overall size range from 13 nm to 764.7 nm.

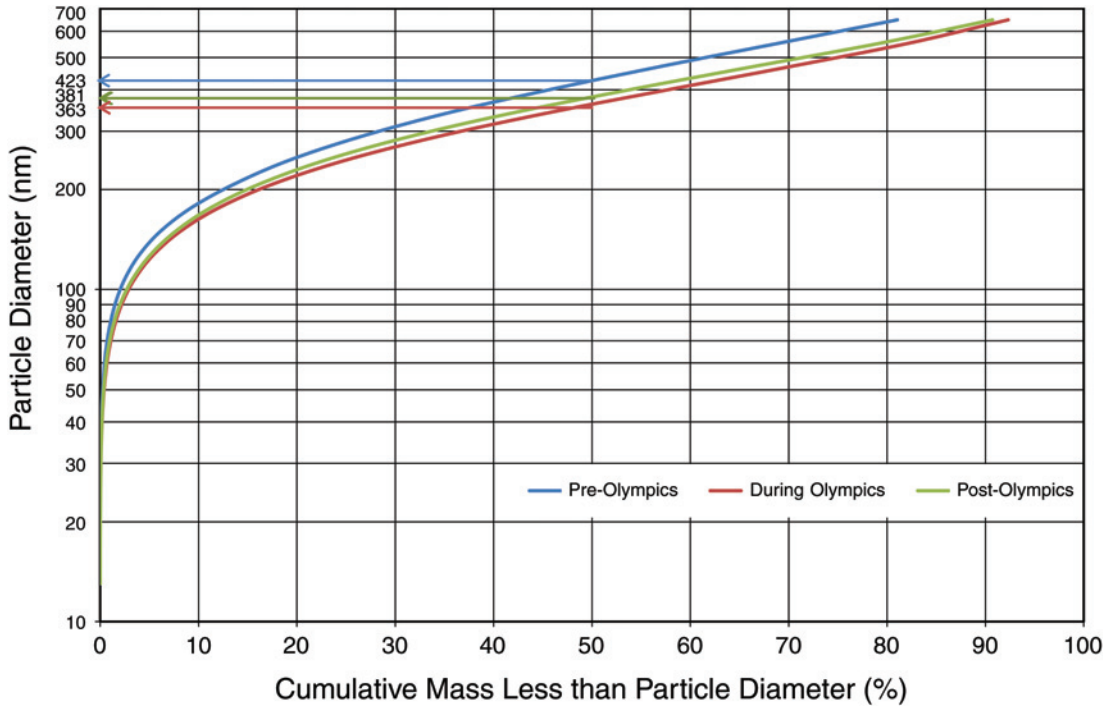

Figure 8. PM size distribution plot used to calculate the mass median particle diameter for each of the three periods. The mass concentrations of the 26 size bins were calculated for each of the three Olympic periods (the particle density was assumed to be 1 g/cm^{-3}). The cumulative percent mass less than the diameter on the lower side of each size bin was calculated and then plotted versus the diameter.

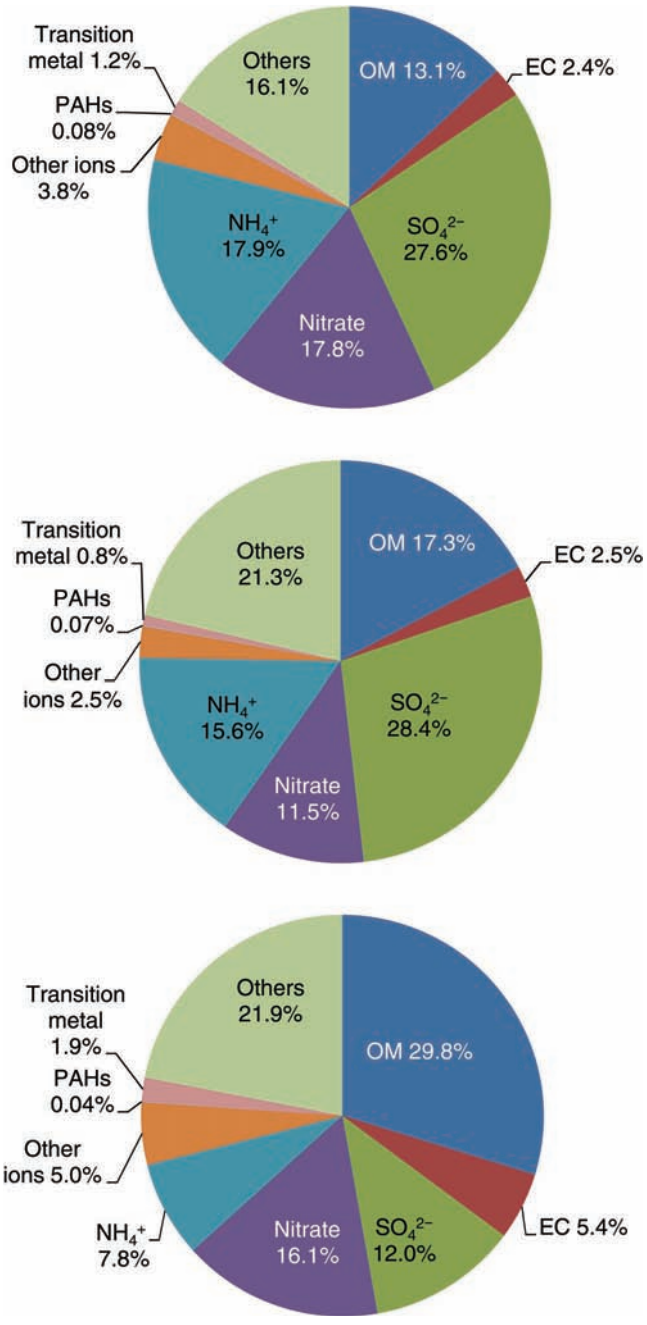

Figure 9. Mean fractional contributions (%) of species or species category to $PM_{2.5}$ mass: (top) the pre-Olympics period; (middle) the during-Olympics period; (bottom) the post-Olympics period. In the diagrams, OM equals 1.4 × OC; transition metals include Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Cd, Th, and U; PAHs include acenaphthylene, fluorene, phenanthrene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*e*]pyrene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenzo[*a*,*h*]anthracene, and benzo[*g*,*h*,*i*]perylene; other ions include Na^+, K^+, Mg^{2+}, Ca^{2+}, F^-, and Cl^-.

and further increased to 29.8% in the post-Olympics period. The EC fraction was similar in the pre-Olympics period (2.4%) and the during-Olympics period (2.5%) but was substantially higher in the post-Olympics period (5.4%). The SO_4^{2-} fraction was similar in the pre-Olympics period (27.6%) and the during-Olympics period (28.4%) but was substantially lower in the post-Olympics period (12.0%). The pattern for NH_4^+ was the same as for SO_4^{2-}: the pre-Olympics NH_4^+ fraction (17.9%) was similar to the during-Olympics fraction (15.6%) and higher than the post-Olympics value (7.8%). The NO_3^- fraction was similar in the pre-Olympics (17.8%) and the post-Olympics period (16.1%) and was lower in the during-Olympics period (11.5%). Other ions contributed a relatively small fraction to $PM_{2.5}$ mass: 3.8%, 2.5%, and 5.0% in the pre-, during-, and post-Olympics periods, respectively. The PAH fraction in the pre-Olympics period (0.08%) was similar to that in the during-Olympics period (0.07%); the post-Olympics PAH fraction (0.04%) was lower. The fraction of transition metals was lowest in the during-Olympics period (0.8%) compared with the pre-Olympics (1.2%) and post-Olympics (1.9%) periods.

Relationships Among Pollutants, Temperature, and Relative Humidity

We assessed relationships among the pollutants, ambient temperature, and RH using Spearman correlation coefficients (see Table 5). As expected, the correlations between $PM_{2.5}$ mass and some of its constituents (SO_4^{2-}, EC, and OC) were high (r ranging from 0.67 to 0.90), as were the correlations between $PM_{2.5}$ mass and SO_2 (r = 0.74) and between $PM_{2.5}$ mass and CO (r = 0.69). In contrast, the correlations of $PM_{2.5}$ mass to the following were relatively weaker: NO_2 (r = 0.42), TPN (r = 0.11), O_3 (r = 0.12), O_3 max (r = 0.22), temperature (r = 0.18), and RH (r = 0.33).

Ambient temperature had relatively higher correlations with NO_2 (r = −0.59, inversely), O_3 (r = 0.72), O_3 max (r = 0.71), and SO_4^{2-} (r = 0.47), whereas RH had relatively higher correlations with SO_4^{2-} (r = 0.50) and TPN inversely (r = −0.54). Among the gaseous pollutants, SO_2 was positively correlated with CO (r = 0.58) and with NO_2 (r = 0.64); CO was positively correlated with NO_2 (r = 0.53); NO_2 was negatively correlated with O_3 (r = −0.58) and O_3 max (r = −0.40); and O_3 was highly positively correlated with O_3 max (r = 0.91).

BIOMARKER LEVELS BEFORE, DURING, AND AFTER THE OLYMPICS

Period-specific means and standard errors (SEs), accounting for the repeated measures using mixed-effects models with adjustments for covariates, are shown in

Table 5. Spearman Correlation Coefficients for Selected Air Pollutants, Ambient Temperature, and RH[a]

	$PM_{2.5}$ mass (n = 100)	SO_4^{2-} (n = 92)	EC (n = 94)	OC (n = 94)	TPN (n = 93)	SO_2 (n = 91)	CO (n = 100)	NO_2 (n = 100)	O_3 (n = 100)	O_3 max (n = 100)	TEMP[b] (n = 99)	RH (n = 99)
$PM_{2.5}$ mass	1											
SO_4^{2-}	0.90[c]	1										
EC	0.67[c]	0.40[c]	1									
OC	0.69[c]	0.44[c]	0.92[c]	1								
TPN	0.11	−0.21	0.56[c]	0.53[c]	1							
SO_2	0.74[c]	0.63[c]	0.71[c]	0.72[c]	0.40[c]	1						
CO	0.69[c]	0.57[c]	0.58[c]	0.55[c]	0.27[c]	0.58[c]	1					
NO_2	0.42[c]	0.12	0.80[c]	0.75[c]	0.63[c]	0.64[c]	0.53[c]	1				
O_3	0.12	0.35[c]	−0.30[d]	−0.22[d]	−0.33[c]	0.02	−0.13	−0.58[c]	1			
O_3 max	0.22[d]	0.37[c]	−0.12	−0.02	−0.15	0.13	0.01	−0.40[c]	0.91[c]	1		
TEMP	0.18	0.47[c]	−0.27[c]	−0.16	−0.34[c]	−0.07	0.03	−0.59[c]	0.72[c]	0.71[c]	1	
RH	0.33[c]	0.50[c]	−0.14	−0.11	−0.54[c]	−0.06	0.33[c]	−0.11	−0.12	−0.15	0.19	1

[a] All measured on a 24-hr basis except O_3 max, which is the maximum 1-hr average concentration within a 24-hr period.
[b] TEMP indicates ambient temperature.
[c] $P < 0.01$.
[d] $P < 0.05$.

Table 6. In the 125 young participants, who were free of any chronic diseases, we observed decreases, from the pre-Olympics to the during-Olympics period, in mean concentrations or the fraction of above-detection values for all pulmonary biomarkers of respiratory inflammation and oxidative stress (FeNO, EBC nitrate, nitrite, nitrite+ nitrate, and 8-isoprostane) except for an increase in EBC pH (corresponding to a decrease in hydrogen ion concentration) (Table 6). A decrease was also seen in HR, some HRV indices (LF, LF/HF, and VLF), and both diastolic and systolic BP. For systemic inflammation and oxidative stress biomarkers, we observed a decrease in the number of CRP values above the detection limit and in urinary 8-OHdG concentration from the pre- to the during-Olympics period. For hemostasis biomarkers, we observed decreases in concentrations of sCD62P, sCD40L, and vWF. These changes were all salutary based on the hypothesized mechanisms of PM action. Most of the biomarkers increased in the post-Olympics period over the during-Olympics mean levels, with the exception of EBC hydrogen ion, LF, LF/HF, rMSSD, SDNN, total power, CRP, RBCs, WBCs, lymphocytes, platelet aggregation, and vWF.

After adjustment for ambient temperature, RH, sex, and day of the week, the pre-Olympics to during-Olympics changes (%) are shown in Figure 10A. For the biomark ers of autonomic dysfunction, we observed a statistically significant change in HR (−1.7% [95% CI, −3.4 to −0.1]) and marginally significant change in SBP (−1.6 mmHg, or −1.8% [95% CI, −3.9 to 0.4]). For the biomarkers of systemic inflammation and oxidative stress, we observed a nonsignificant increase in WBC count (2.2% [95% CI, −2.3 to 6.6]), a marginally significant increase in lymphocytes (2.2% [95% CI, −0.3 to 4.7]), and a large and statistically significant decrease in 8-OHdG (−58.3% [95% CI, −72.5 to −36.7]). For the biomarkers of pulmonary inflammation and oxidative stress, we observed large and statistically significant decreases in FeNO (−60.3% [95% CI, −66.0 to −53.6]), EBC nitrite (−30.0% [95% CI, −39.3 to −19.3]), EBC nitrate (−21.5% [95% CI, −35.5 to −4.5]), and EBC nitrite+nitrate (−17.6% [95% CI, −28.4 to −5.1]) and a statistically significant increase in EBC pH (3.5% [95% CI, 2.2 to 4.9]), corresponding to a large decrease (−46%) in hydrogen ions. For the biomarkers of hemostasis, we observed a large and statistically significant decrease in sCD62P (−34% [95% CI, −38.4 to −29.2]), a smaller but significant decrease in sCD40L (−5.7% [95% CI, −10.5 to −0.7]), a moderate and significant decrease in vWF (−13.1% [95% CI, −18.6 to −7.5]), and a significant increase in platelet aggregation (7.4% [95% CI, 2.2 to 12.5]). The biomarkers not listed here exhibited either a highly nonsignificant (with a large 95% CI) change or a near-zero change.

Table 6. Period-Specific Means and Percent Changes for Biomarker Measurements Based on Period Estimates from Mixed-Effects Models, Accounting for Repeated Measures and Controlling for Temperature and RH

Biomarker	Pre-Olympics Mean ± SE	During Olympics Mean ± SE	Post-Olympics Mean ± SE	Pre–During Olympics Percent Change (95% CI)	During–Post-Olympics Percent Change (95% CI)
Autonomic Dysfunction					
HR (bpm)[a]	66.5 ± 1.0	65.4 ± 1.0	66.1 ± 1.0	−1.7 (−3.4 to −0.1)	1.1 (−2.5 to 4.9)
HF (ms^2)[a]	557.9 ± 1.1	583.5 ± 1.1	590.8 ± 1.1	4.6 (−10.5 to 22.3)	1.2 (−28.9 to 44.2)
LF (ms^2)[a]	459.6 ± 1.1	446.3 ± 1.1	407.6 ± 1.1	−2.9 (−19.0 to 16.4)	−8.7 (−36.6 to 31.6)
LF/HF[a]	0.87 ± 1.1	0.78 ± 1.1	0.69 ± 1.1	−10.6 (−24.5 to 5.8)	−10.7 (−37.3 to 27.3)
rMSSD (ms)[a]	56 ± 1.1	59 ± 1.1	46 ± 1.1	6.14 (−5.6 to 19.4)	−22.0 (−40.2 to 1.9)
SDNN (ms)[a]	62 ± 1.0	63 ± 1.1	54 ± 1.0	0.8 (−7.5 to 9.8)	−13.6 (−28.9 to 4.9)
VLF (ms^2)[a]	623.9 ± 1.1	553.9 ± 1.1	725.4 ± 1.1	−11.2 (−25.2 to 5.4)	31.0 (−10.8 to 92.3)
Total power (ms^2)[a]	1883.4 ± 1.1	1943.1 ± 1.1	1878.2 ± 1.1	3.2 (−10.8 to 19.4)	−3.3 (−28.2 to 30.2)
DBP (mmHg)	60.2 ± 1.2	60.1 ± 1.6	60.1 ± 2.0	−0.3 (−3.0 to 2.5)	0.1 (−9.7 to 9.9)
SBP (mmHg)	102.5 ± 1.4	100.9 ± 1.8	110.5 ± 2.3	−1.8 (−3.9 to 0.4)	10.7 (2.8 to 18.6)
Systemic Inflammation and Oxidative Stress					
Fibrinogen (g/L)	2.50 ± 0.04	2.50 ± 0.05	2.61 ± 0.06	−0.1 (−2.5 to 2.2)	4.3 (−1.7 to 10.2)
CRP (% ≥ 0.3 mg/L)	55	46	36	—	—
RBCs ($\times 10^{12}$/L)	4.57 ± 0.03	4.61 ± 0.03	4.48 ± 0.03	0.9 (0.3 to 1.5)	−2.7 (−3.8 to −1.6)
WBCs ($\times 10^9$/L)	5.29 ± 0.13	5.40 ± 0.15	5.21 ± 0.16	2.2 (−2.3 to 6.6)	−3.9 (−11.5 to 3.6)
Lymphocytes ($\times 10^9$/L)	1.66 ± 0.03	1.70 ± 0.04	1.59 ± 0.04	2.2 (−0.3 to 4.7)	−6.9 (−11.4 to −2.5)
Neutrophils ($\times 10^9$/L)	3.03 ± 0.09	3.06 ± 0.12	3.19 ± 0.14	1.0 (−5.4 to 7.3)	4.7 (−7.7 to 17.0)
Urinary 8-OHdG (mg/mol creatinine)[a]	2.16 ± 1.81	0.90 ± 1.95	3.74 ± 1.46	−58.3 (−72.5 to −36.7)	315 (62.0 to 962)
Pulmonary Oxidative Stress and Inflammation					
FeNO (ppb)[a]	11.53 ± 1.07	4.58 ± 1.10	10.52 ± 1.13	−60.3 (−66.0 to −53.6)	130 (62.5 to 225)
EBC					
Nitrite (μM)[a]	6.30 ± 1.06	4.41 ± 1.09	11.43 ± 1.15	−30.0 (−39.3 to −19.3)	159 (71.8 to 292)
Nitrate (μM)[a]	2.84 ± 1.08	2.23 ± 1.13	5.82 ± 1.22	−21.5 (−35.5 to −4.5)	161 (48.0 to 362)
Nitrite+nitrate (μM)[a]	10.11 ± 1.06	8.34 ± 1.09	18.70 ± 1.14	−17.6 (−28.4 to −5.1)	124 (50.9 to 233)
pH	7.41 ± 0.07	7.68 ± 0.10	7.29 ± 0.14	3.5 (2.2 to 4.9)	−4.8 (−9.4 to −0.2)
8-Isoprostane (% ≥ 1.56 pg/mL)	68	44	74	—	—
Hemostasis					
sCD62P (ng/mL)[a]	6.29 ± 1.03	4.16 ± 1.04	5.56 ± 1.04	−34.0 (−38.4 to −29.2)	33.7 (17.7 to 51.8)
sCD40L (ng/mL)[a]	1.86 ± 1.02	1.76 ± 1.03	1.92 ± 1.04	−5.7 (−10.5 to −0.7)	9.1 (−3.7 to 23.5)
Platelet aggregation (%)	69.29 ± 3.45	76.93 ± 4.74	31.65 ± 6.32	7.4 (2.2 to 12.5)	−40.8 (−51.0 to −30.6)
vWF (%)	106.4 ± 4.0	92.6 ± 5.1	79.5 ± 6.4	−13.1 (−18.6 to −7.5)	−14.2 (−29.9 to 1.6)

[a] Biomarker had skewed data distributions, for which geometric means were reported. Dash indicates no calculations for percent changes between periods were done due to insufficient detection levels.

The during-Olympics to post-Olympics covariate-adjusted changes (%) are shown in Figure 10B. For the biomarkers of autonomic dysfunction, we observed statistically nonsignificant reductions in LF (−8.7% [95% CI, −36.6 to 31.6]), LF/HF (−10.7% [95% CI, −37.3 to 27.3]), and SDNN (−13.6% [95% CI, −28.9 to 4.9]); a marginally significant decrease in rMSSD (−22.0 [95% CI, −40.2 to 1.9]); a nonsignificant increase in VLF (31.0% [95% CI, −10.8 to 92.3]); and a significant increase in SBP (10.7% [95% CI, 2.8 to 18.6]). For the biomarkers of systemic inflammation and oxidative stress, we observed nonsignificant increases in fibrinogen (4.3% [95% CI, −1.7 to 10.2]) and neutrophil count (4.7% [95% CI, −7.7 to 17.0]), a nonsignificant decrease in WBC count (−3.9% [95% CI, −11.53 to 3.6]), a significant decrease in lymphocytes (−6.9% [95% CI, −11.4 to −2.5]), and a very large and significant increase in 8-OHdG (315% [95% CI, 62.0 to 962]). For the biomarkers of pulmonary inflammation and oxidative stress, we observed large and significant increases in FeNO (130% [95% CI, 62.5 to 225]), EBC nitrite (159%

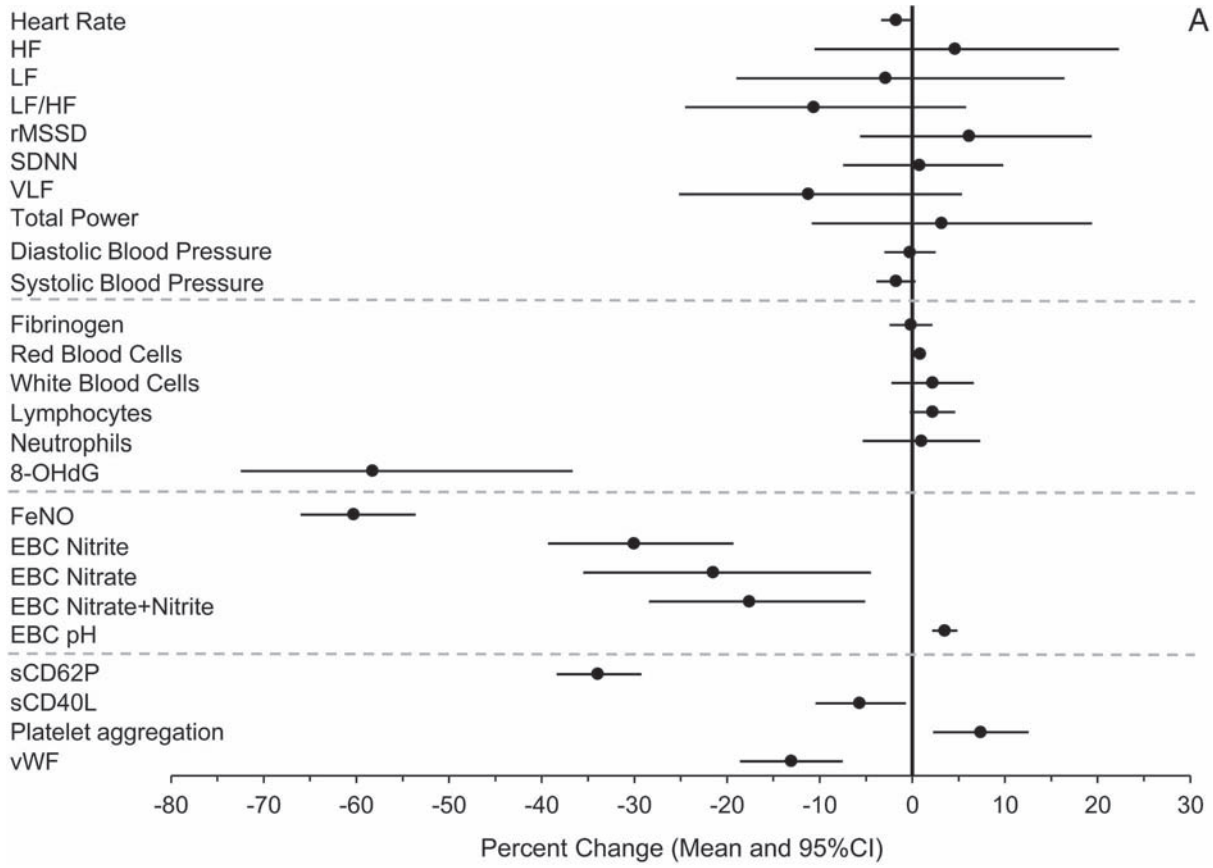

Figure 10. Estimated means and 95% confidence intervals for percent changes in biomarker levels, adjusted for ambient temperature, RH, sex, and day of the week: (A) from the pre-Olympics to the during-Olympics period; (B) from the during-Olympics to the post-Olympics period. (Note different scales for **A** and **B**.)

(*Figure continues on next page*)

[95% CI, 71.8 to 292]), EBC nitrate (161% [95% CI, 48.0 to 362]), and EBC nitrite+nitrate (124% [95% CI, 50.9 to 233]); and a significant decrease in EBC pH (−4.8% [95% CI, −9.4 to −0.2]), corresponding to a large increase (146%) in hydrogen ions. For the biomarkers of hemostasis, we observed a large and significant increase in sCD62P (33.7% [95% CI, 17.7 to 51.8]), a smaller and marginally significant increase in sCD40L (9.1% [95% CI, −3.7 to 23.5]), a marginally significant decrease in vWF (−14.2% [95% CI, −29.9 to 1.6]), and a significant decrease in platelet aggregation (−40.8% [95% CI, −51.0 to −30.6]).

Biomarker levels, unadjusted for covariates and for the repeated-measure structure using raw data, are summarized by period in Appendix B. Intra-class correlations for each biomarker are presented by period in Appendix I. Among all the measured biomarkers, the following had skewed data distributions: FeNO, nitrite, nitrate, nitrite+nitrate, all HRV indices (SDNN, rMSSD, LF, HF, and total power), and sCD40L. About 13% of all urinary samples had 8-OHdG concentrations below the detection limit, and the data distribution was highly skewed. EBC 8-isoprostane and CRP each had a large percentage of nondetectable values.

Biomarker–Pollutant Relationships

For each biomarker, we plotted the point estimates and 95% confidence intervals of the percent changes in biomarker per IQR increase in pollutant concentration. The IQR values used for estimating pollutant-specific effects are shown in Table 7.

Biomarkers of Autonomic Dysfunction

Heart Rate As shown in Figure 11, we observed positive associations of HR with all pollutants except O_3 for most lag days, although statistical significance was observed

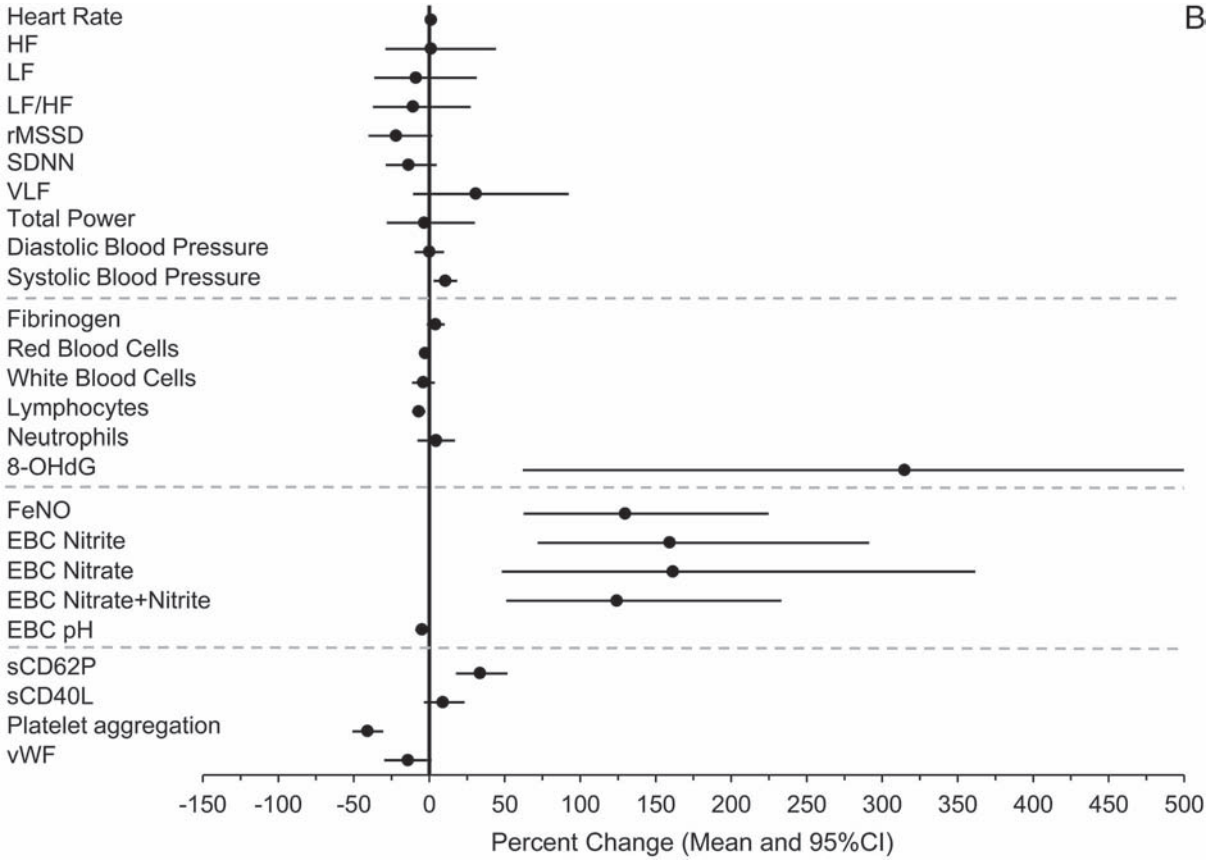

Figure 10 (*Continued*).

only for $PM_{2.5}$, SO_4^{2-}, and SO_2, all at lag day 1, and for EC and TPN at lag day 3. The largest effect estimates corresponding to these lag days were 1.5% for $PM_{2.5}$, 2.2% for SO_4^{2-}, 0.9% for EC, 0.5% for TPN, and 1.1% for SO_2. These findings are, in general, consistent with the hypothesis that air pollution can lead to increased HR.

Heart Rate Variability Similar to the HR observations, VLF appeared to increase as pollutant concentration increased for most lag days and for all pollutants except O_3; statistical significance was seen for SO_4^{2-} at lag 4 and EC at lag 5 (see Figure 12). The pattern was less clear for SDNN (see Figure 13), as associations bounced back and forth between the negative and positive, with statistical significance observed only for $PM_{2.5}$ (at lag day 5, positive), SO_4^{2-} (at lag days 4 and 5, positive), OC (at lag days 1 and 2, negative), and NO_2 (at lags 0 and 1, negative). The pattern for rMSSD (see Figure 14) was even more complex, as we observed significant positive associations with $PM_{2.5}$ at lag day 5 and with SO_4^{2-} at lag days 4 and 5, while seeing significant negative associations with EC and OC at lag days 0 to 2, with OC at lag day 3, with TPN

Table 7. Interquartile Range Values Used for Estimating Pollutant-Specific Effects

	$PM_{2.5}$ ($\mu g/m^3$)	SO_4^{2-} ($\mu g/m^3$)	EC ($\mu g/m^3$)	OC ($\mu g/m^3$)	TPN ($/cm^3$)	SO_2 (ppb)	CO (ppm)	NO_2 (ppb)	O_3 (ppb)
IQR	76.8	28.0	1.4	5.1	6572	5.4	0.65	18.7	25.4

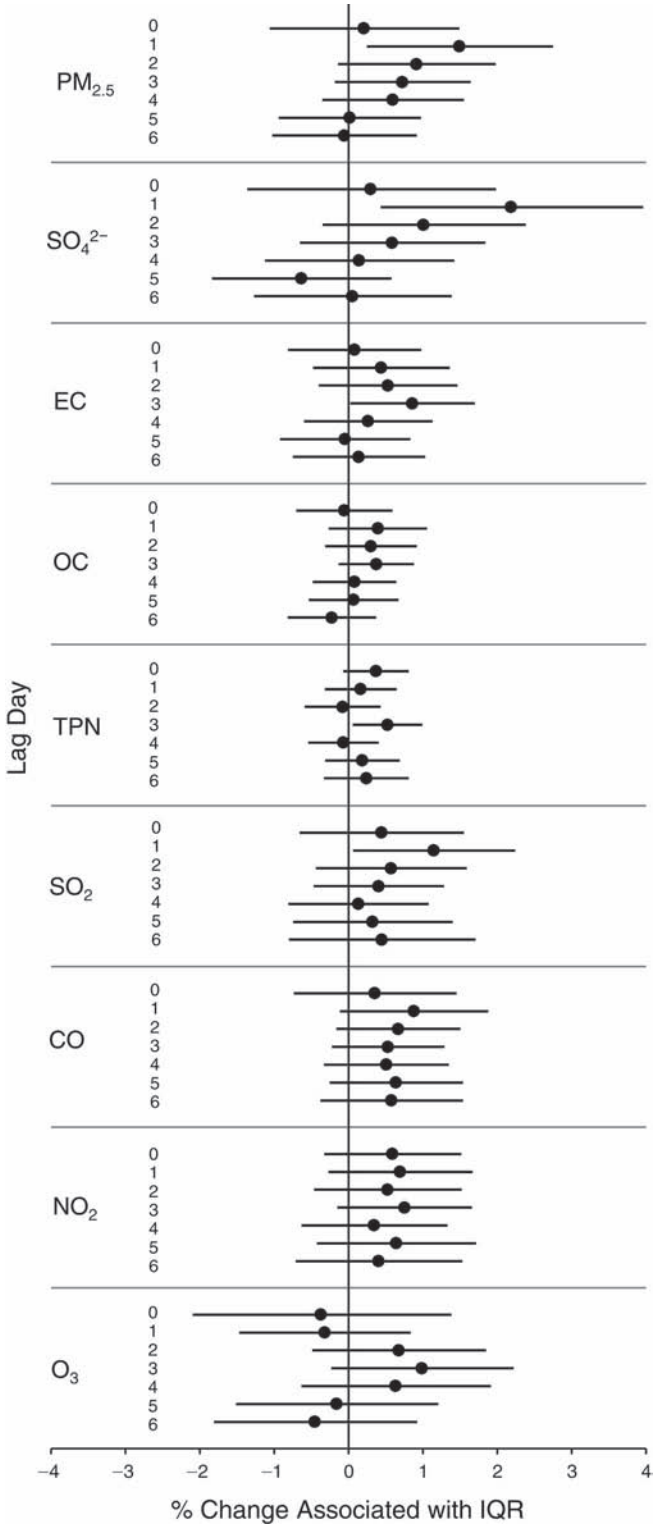

Figure 11. Estimated means and 95% CIs for the percent change in HR associated with one IQR increase in pollutant concentration, controlling for temperature (df in natural spline = 1), RH (df = 1), sex, and day of the week for biomarker measurements.

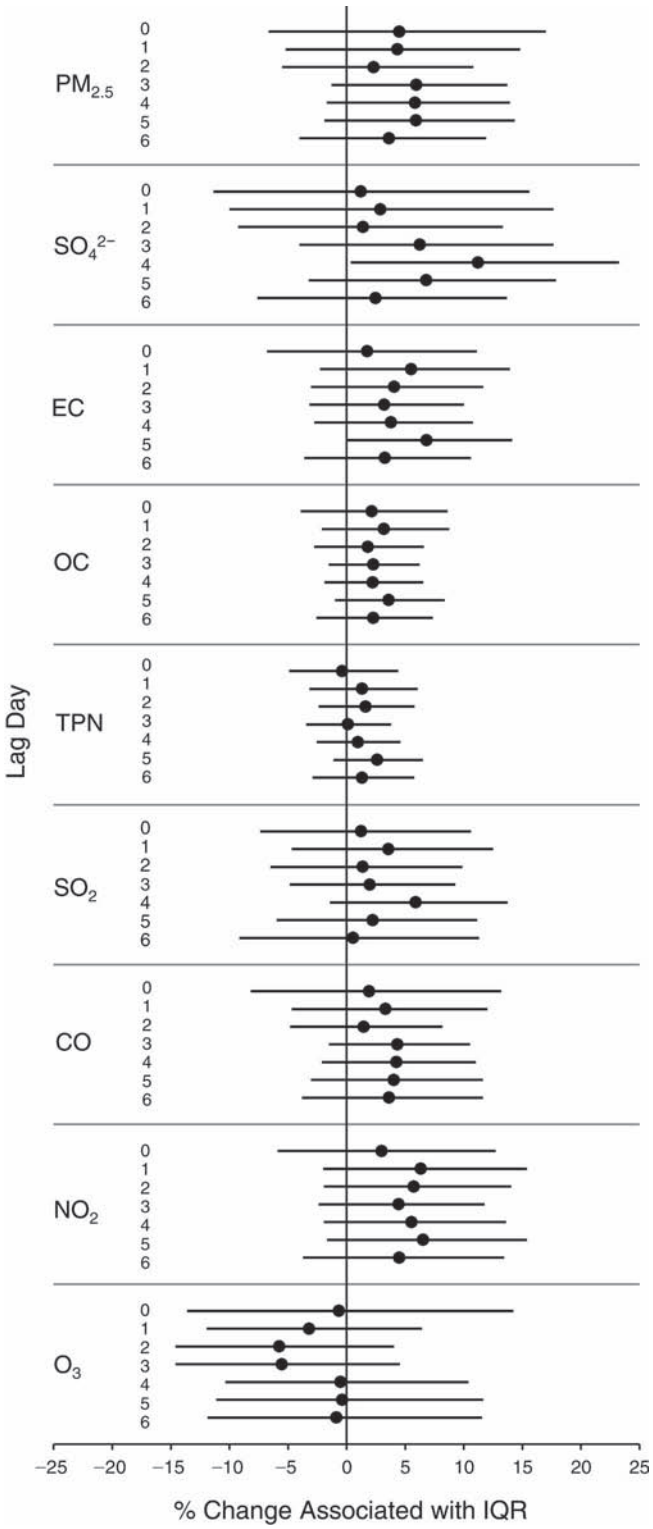

Figure 12. Estimated means and 95% CIs for the percent change in VLF (HRV) associated with one IQR increase in pollutant concentration, controlling for temperature (df in natural spline = 1), RH (df = 1), 7-day moving average of temperature (df = 1), sex, and day of the week for biomarker measurements.

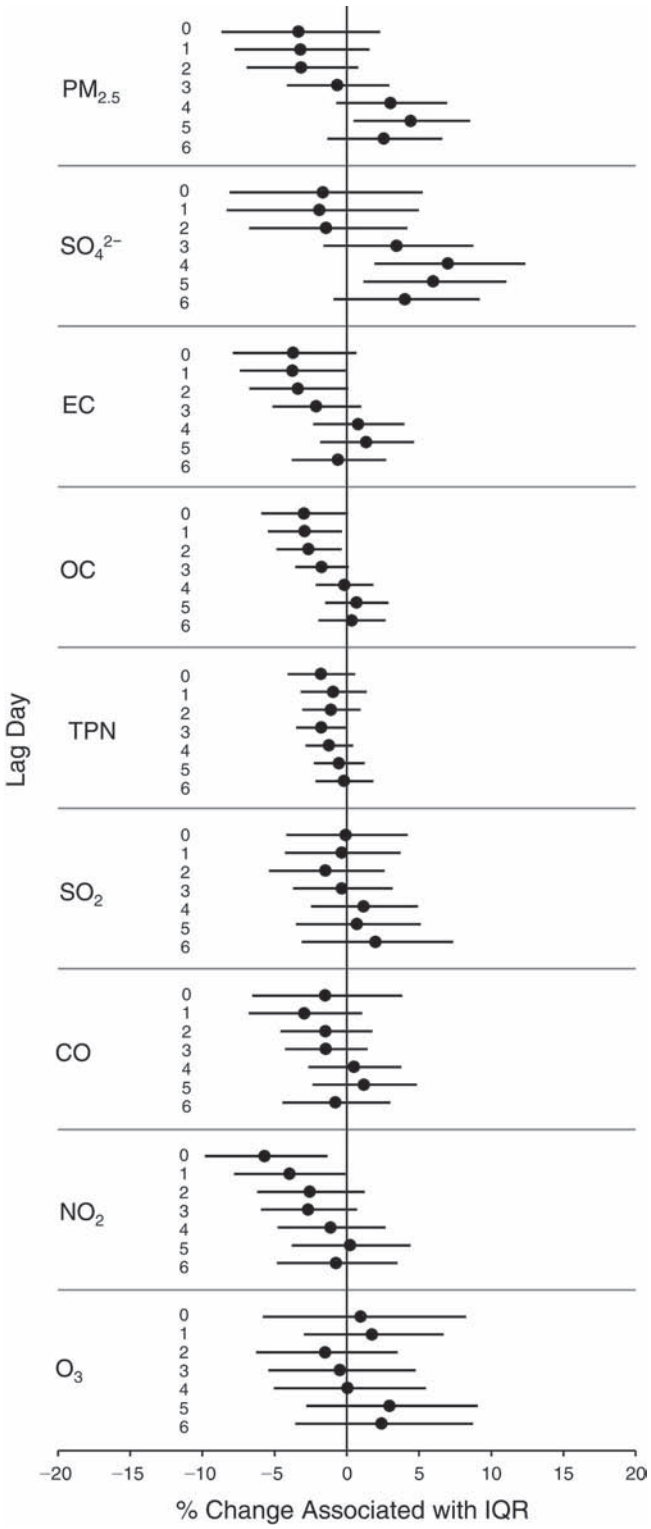

Figure 13. Estimated means and 95% CIs for the percent change in SDNN (HRV) associated with one IQR increase in pollutant concentration, controlling for temperature (df in natural spline = 1), RH (df = 1), 7-day moving average of temperature (df = 1), sex, and day of the week for biomarker measurements.

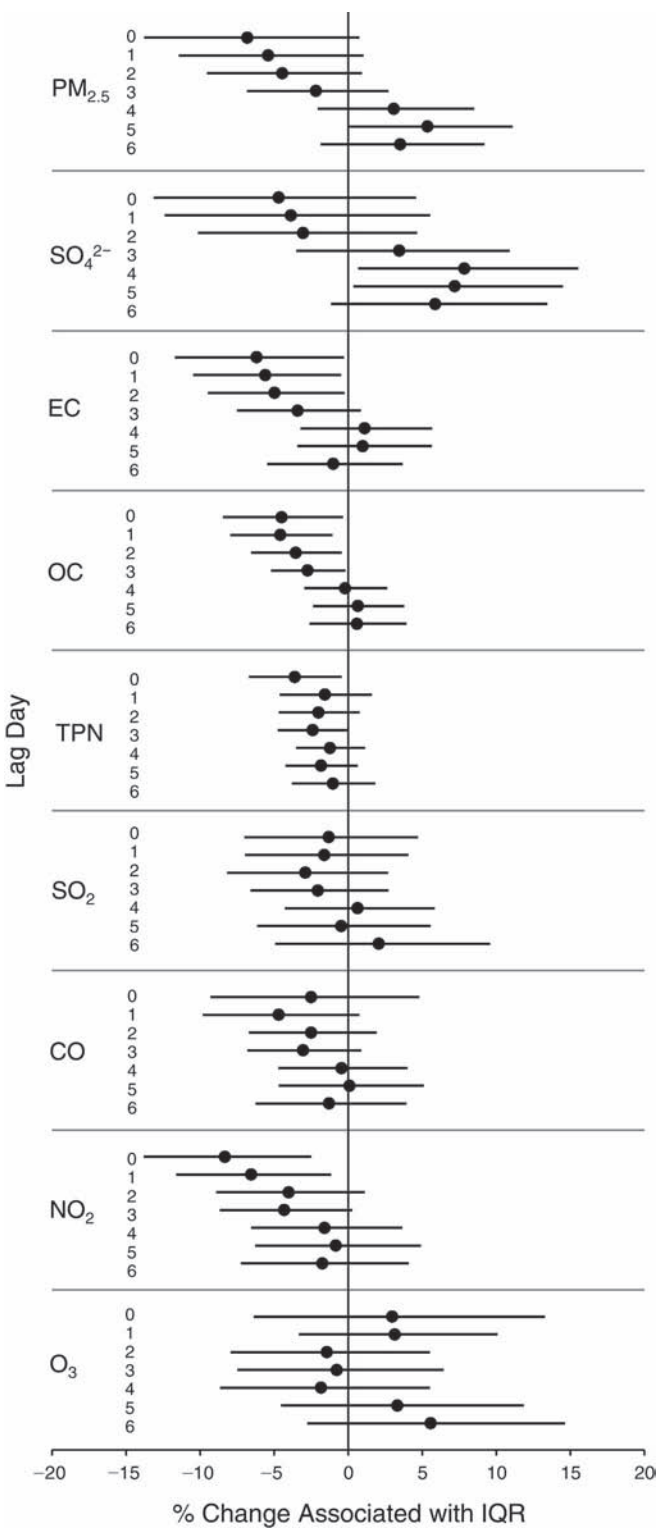

Figure 14. Estimated means and 95% CIs for the percent change in rMSSD (HRV) associated with one IQR increase in pollutant concentration, controlling for temperature (df in natural spline = 1), RH (df = 1), 7-day moving average of temperature (df = 1), sex, and day of the week for biomarker measurements.

at lag days 0 and 3, and with NO_2 at lag days 0 and 1. We did not observe a clear pattern of pollutant response with LF, HF, LF/HF, or total power of the HRV indices (plots not shown).

Blood Pressure The relation between SBP and pollutant concentrations (see Figure 15) had a pattern somewhat similar to that for SDNN, as we observed both significant positive and negative associations bouncing back and forth across lag days. We did not observe a clear pattern for DBP (plot not shown).

Biomarkers of Systemic Inflammation and Oxidative Stress

Fibrinogen Consistent with the hypothesis that air pollution exposure increases plasma fibrinogen via systemic inflammation, we observed significant increases (ranging 0.8%–1.9%) in fibrinogen (Figure 16) associated with IQR increases in $PM_{2.5}$, EC, and OC (all at lags 2 and 3), SO_2 (at lags 3 and 6), and NO_2 (at lag day 0). We also observed similarly sized nonsignificant increases associated with IQR increases in SO_4^{2-} and CO at multiple lag days. However, O_3 appeared to have negative, nonsignificant associations with fibrinogen at multiple lag days.

Red Blood Cell Counts As shown in Figure 17, we observed significant negative associations between RBC count and all pollutants except O_3. The largest reductions per IQR increase in pollutant concentration (ranging from 0.4%–1.4%) were observed at earlier lag days (0 and/or 1) for $PM_{2.5}$, SO_4^{2-}, EC, OC, and SO_2, and at later lag days for CO and NO_2. These negative associations are contrary to the hypothesis that increased air pollutant concentrations are associated with increases in RBCs through inflammation. In contrast, for O_3 we observed significant increases in RBC counts (0.8%–1.2%) associated with each IQR increase at multiple lag days.

White Blood Cell Counts As shown in Figure 18 and contrary to the hypothesis, we observed statistically significant decreases of 1.5% to 3.4% in WBCs associated with IQR increases in OC, TPN, SO_2, CO, and NO_2, at lag days 4 to 6. In addition, TPN was positively and significantly associated with WBC count at lag 0, while O_3 was positively and nonsignificantly associated with WBC count at lags 1 to 6.

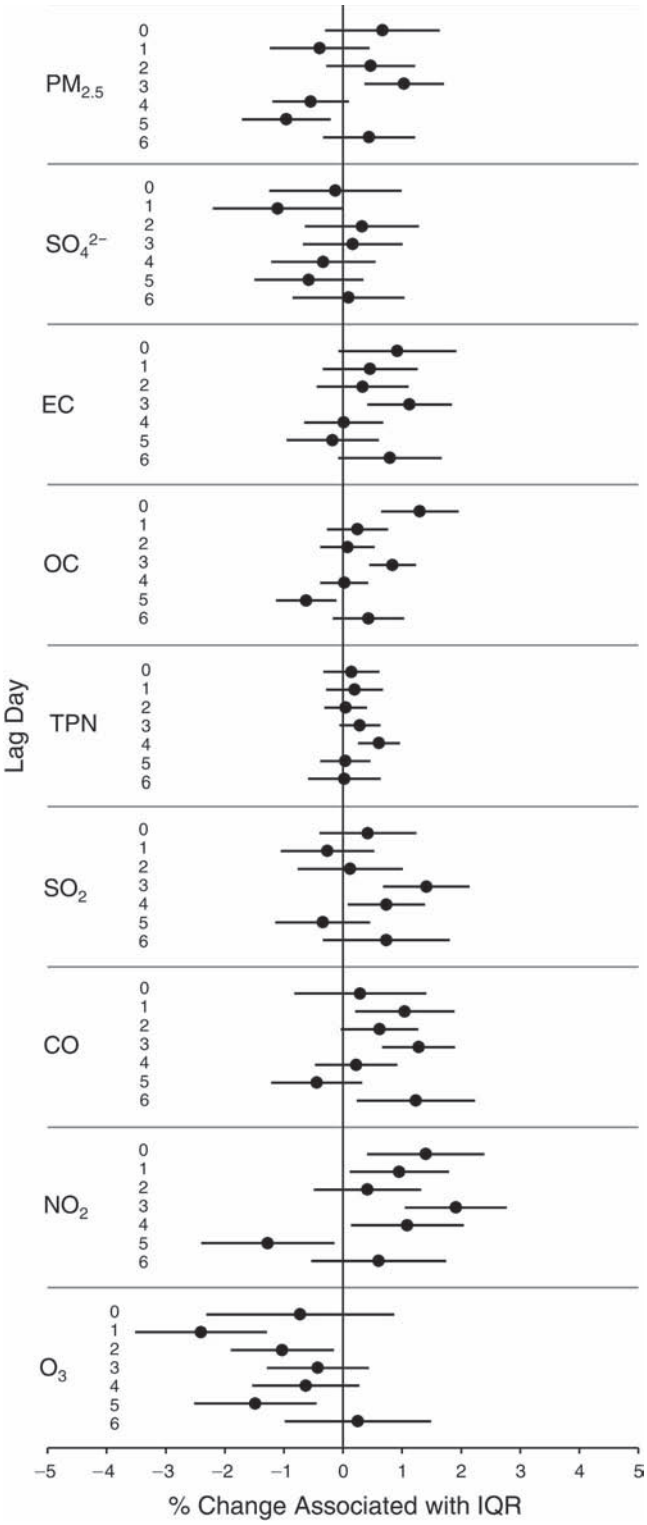

Figure 15. Estimated means and 95% CIs for the percent change in SBP associated with one IQR increase in pollutant concentration, controlling for temperature (df in natural spline = 3), RH (df = 2), 7-day moving average of temperature (df = 3), 2-day moving average of RH (df = 3), sex, and day of the week for biomarker measurements.

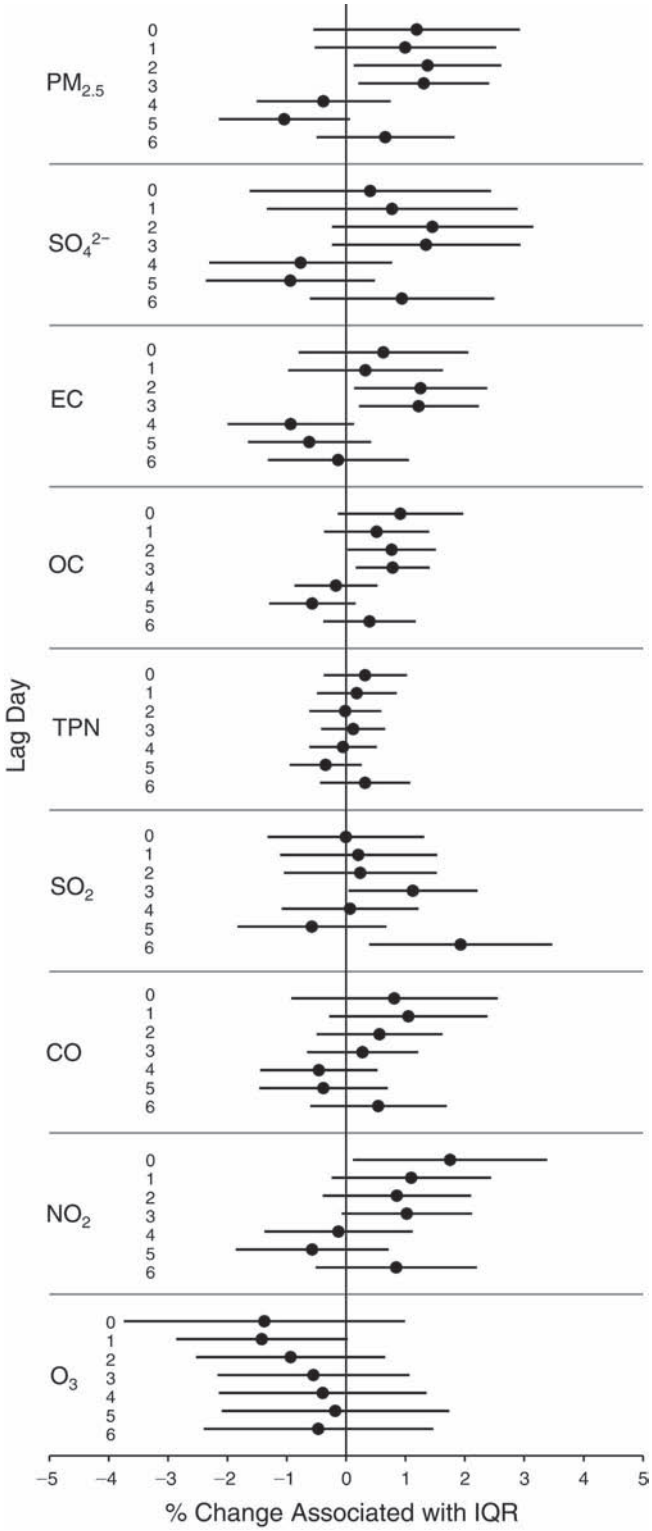

Figure 16. Estimated means and 95% CIs for the percent change in fibrinogen level associated with one IQR increase in pollutant concentration, controlling for temperature (df in natural spline = 3), RH (df = 1), 6-day moving average of temperature (df = 1), sex, and day of the week for biomarker measurements.

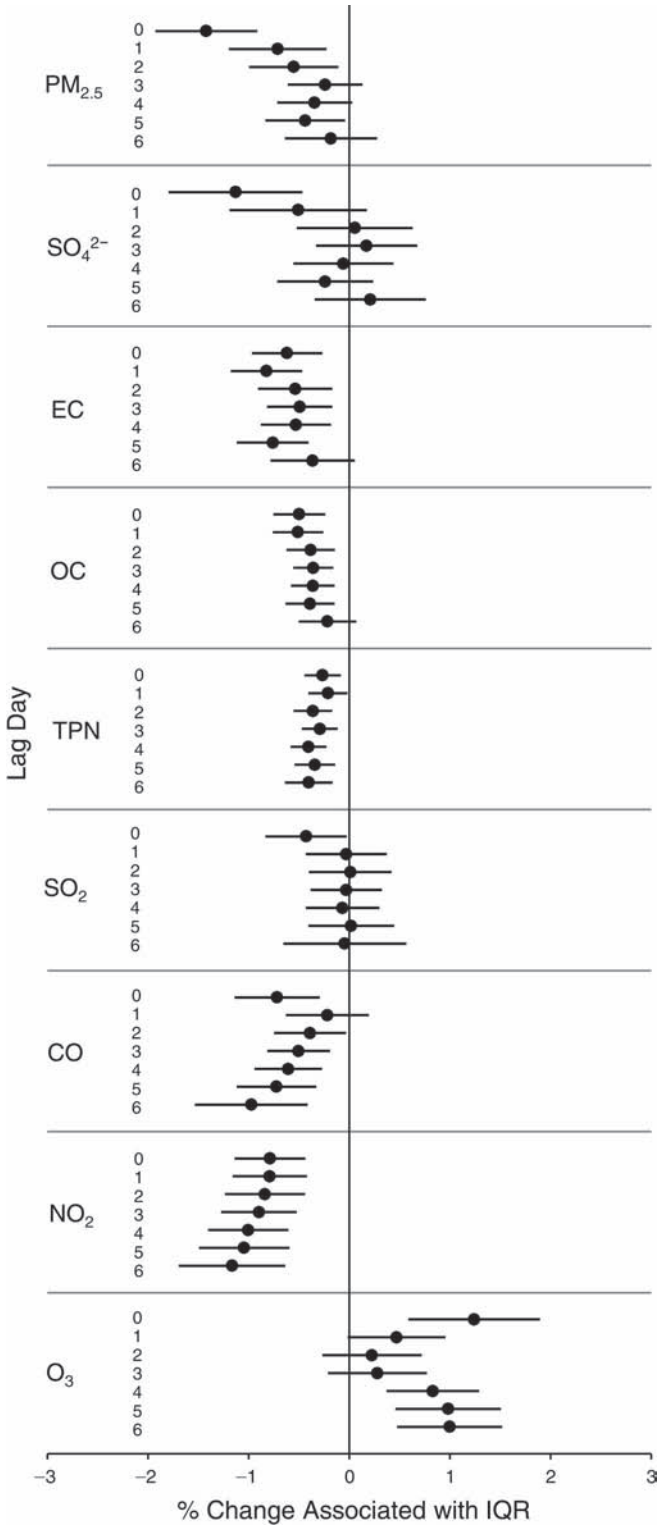

Figure 17. Estimated means and 95% CIs for the percent change in RBC count associated with one IQR increase in pollutant concentration, controlling for temperature (df in natural spline = 1), RH (df = 1), 4-day moving average of RH (df = 1), sex, and day of the week for biomarker measurements.

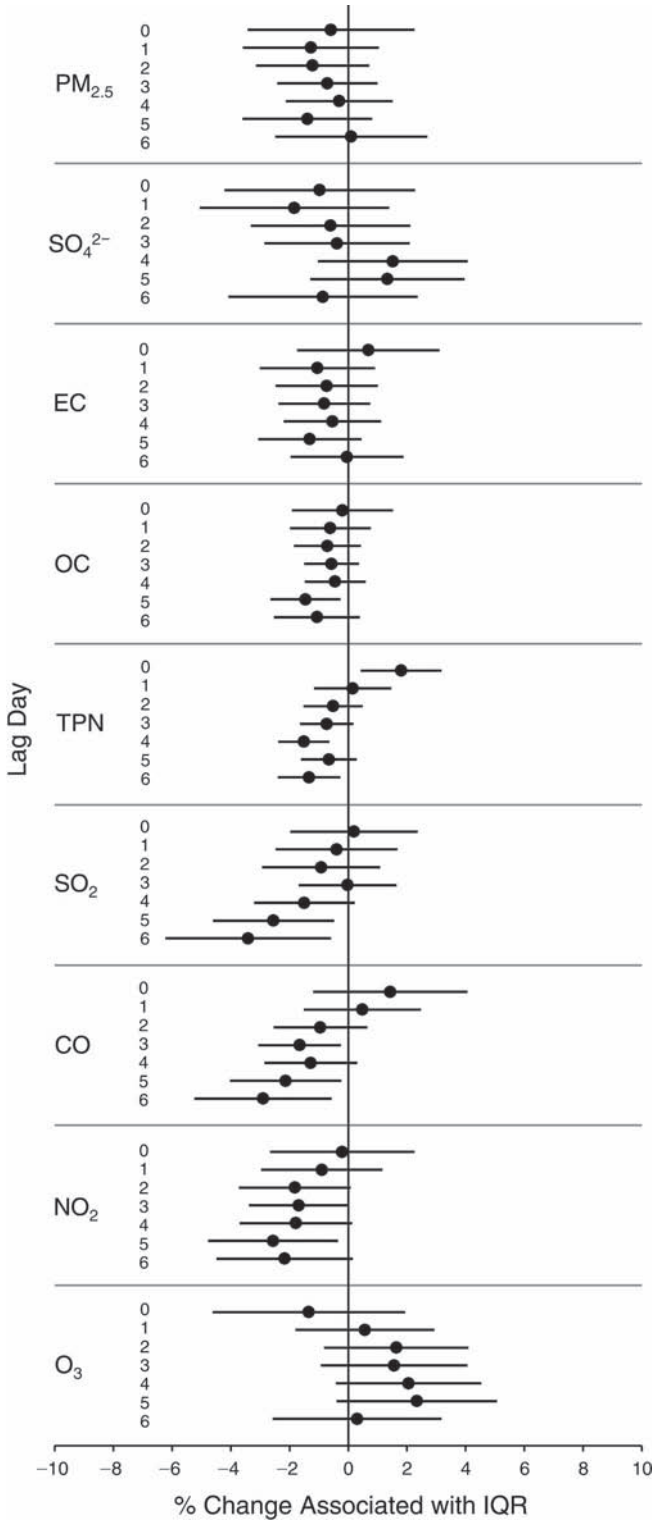

Figure 18. Estimated means and 95% CIs for the percent change in WBC count associated with one IQR increase in pollutant concentration, controlling for temperature (df in natural spline = 1), RH (df = 1), 7-day moving average of temperature (df = 3), 6-day moving average of RH (df = 3), sex, and day of the week for biomarker measurements.

Urinary 8-OHdG As shown in Figure 19, we observed mostly positive and significant associations between 8-OHdG levels and all pollutants except O_3 across lag days, which is consistent with the hypothesis that air pollution exposure leads to increased urinary 8-OHdG concentration, reflecting the increased body burden from oxidative stress. Across lag days, the largest effect estimates per IQR increase in pollutant concentrations were 57.6% for $PM_{2.5}$, 75.4% for SO_4^{2-}, 47.7% for EC, 23.6% for OC, 41.4% for SO_2, 42.2% for CO, and 51.9% for NO_2, all at lag 1, and 24.7% for TPN at lag 3. The largest O_3 negative effect estimate was −30.5% at lag 5.

Lymphocytes and Neutrophils As shown in Figure 20, we observed significant negative associations of lymphocyte concentration (absolute count) with $PM_{2.5}$ at lag day 0, EC at lag days 0 and 1, OC at lag day 1, TPN at lag days 0 and 2, CO at lag day 0, and NO_2 at lag days 0 and 1. In contrast, lymphocyte count was significantly and positively associated with O_3 at lag days 0 and 6. In addition, we observed both nonsignificant increases and decreases associated with increases in pollutant concentration across multiple lag days for each pollutant except NO_2 and TPN (which showed decreases at all lags) and O_3 (which showed increases at all lags). We did not observe a clear pattern for neutrophils in relation to the pollutants across lag days (i.e., nothing showed significance and the associations went in different directions) (plot not shown).

Biomarkers of Pulmonary Inflammation and Oxidative Stress

FeNO As shown in Figure 21, FeNO was significantly and positively associated with $PM_{2.5}$, SO_4^{2-}, EC, and SO_2 at all lag days and with TPN, CO, and NO_2 at most lag days. The effect estimates for most of the pollutants appear to be largest at lag day 0 (except SO_2, which had the largest effect estimate at lag 3). It is generally the case that effect estimates gradually decreased as the lag days increased, at least for the first several lag days. This is even true for O_3, although the direction of the association was negative at lags 0 to 2. However, OC appeared to be a lone exception, as a significant, positive, and small association was observed only at lag day 4, despite the fact that overall the FeNO effect estimates for each IQR increase in pollutant concentration were large compared with the effect estimates for most biomarkers measured in this study. The largest effect estimates across the 7 lag

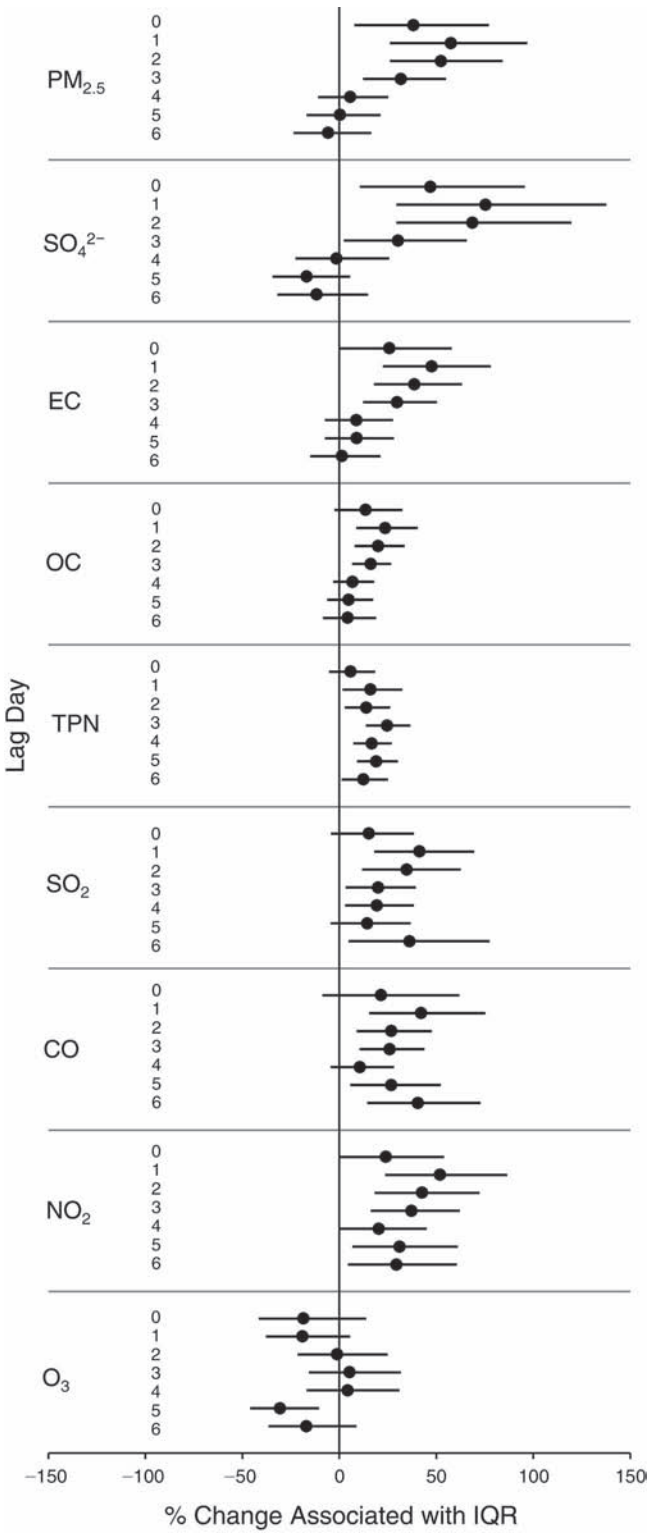

Figure 19. Estimated means and 95% CIs for the percent change in urinary 8-OHdG (creatinine corrected) associated with one IQR increase in pollutant concentration, controlling for temperature (df in natural spline = 1), RH (df = 1), 7-day moving average of temperature (df = 1), 2-day moving average of RH (df = 3), sex, and day of the week for biomarker measurements.

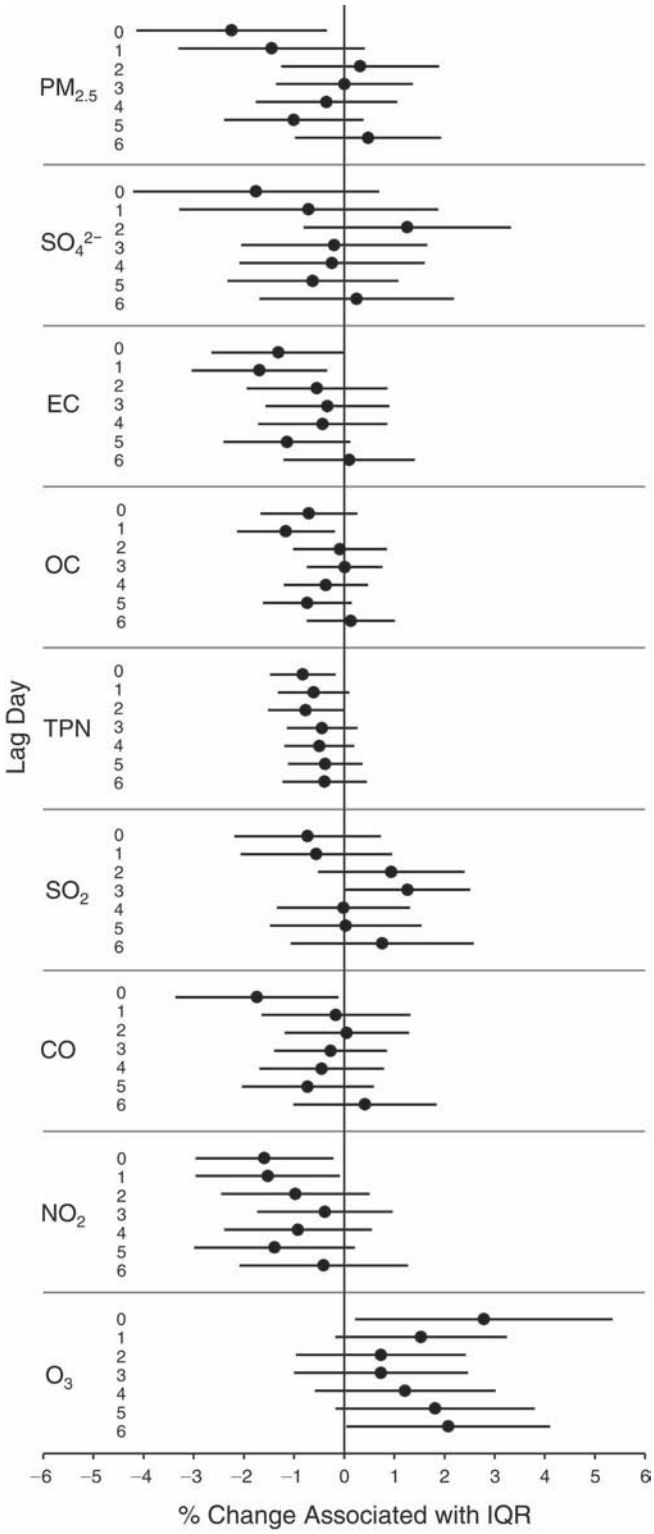

Figure 20. Estimated means and 95% CIs for the percent change in lymphocyte count associated with one IQR increase in pollutant concentration, controlling for temperature (df in natural spline = 1), RH (df = 1), sex, and day of the week for biomarker measurements.

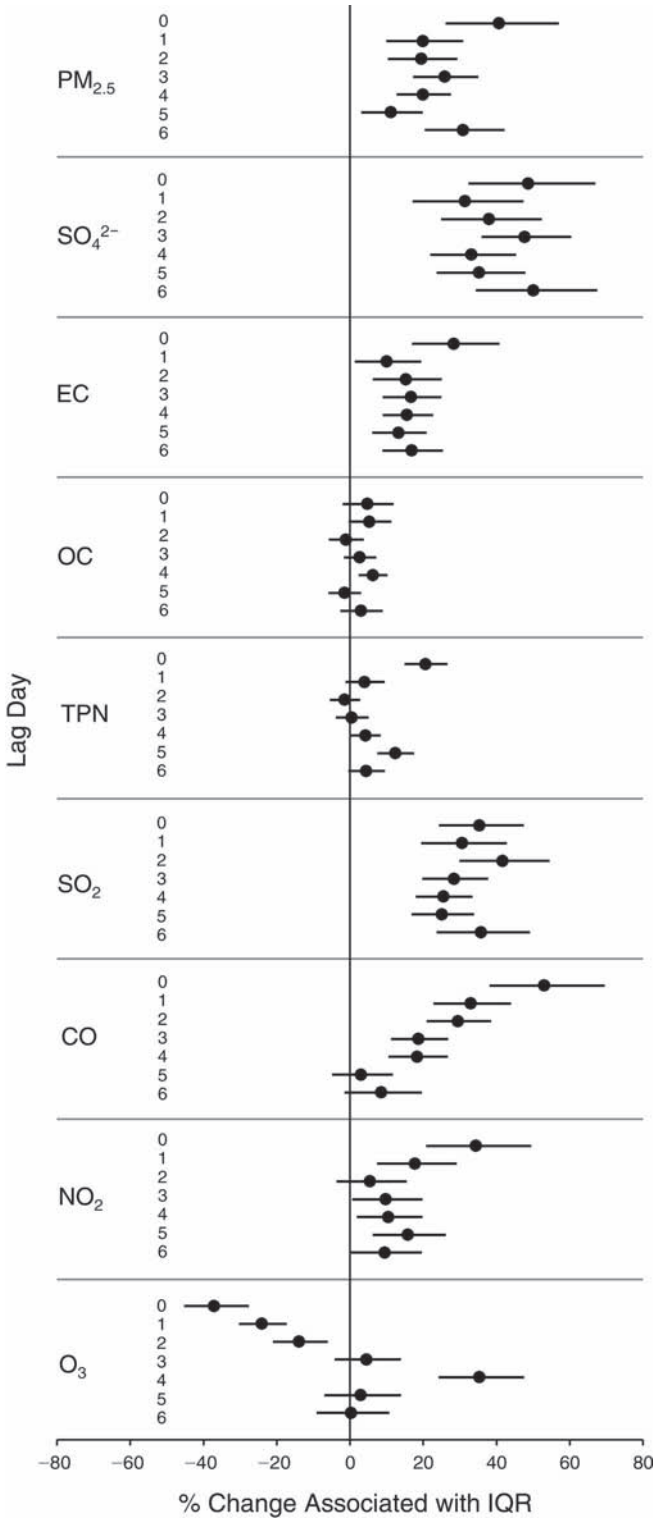

Figure 21. Estimated means and 95% CIs for the percent change in FeNO level associated with one IQR increase in pollutant concentration, controlling for temperature (df in natural spline = 2), RH (df = 3), 7-day moving average of temperature (df = 2), 7-day moving average of RH (df = 3), sex, and day of the week for biomarker measurements.

days were 40.7% for $PM_{2.5}$, 50.0% for SO_4^{2-}, 28.3% for EC, 20.6% for TPN, 41.7% for SO_2, 53.0% for CO, 34.4% for NO_2, and −37.1% for O_3 (in addition, a large positive effect estimate of 35.4% for O_3 was observed at lag 4). The positive FeNO–pollutant associations were in agreement with the hypothesis that pollutant exposure leads to increased FeNO, reflecting increased pulmonary inflammation.

EBC Nitrite, Nitrate, and Nitrite+Nitrate Consistent with the hypothesis, EBC nitrite was positively associated with all pollutants except O_3 at one or more lag days (Figure 22). The effect estimates for $PM_{2.5}$ and its constituents SO_4^{2-}, EC, and OC were largest at lag day 0 and decreased as the lag days increased. This was also the case for TPN for the first 5 lag days, but effect estimates increased from lag 5 to lag 6, when it was the largest. Significant and positive associations were seen for SO_2 at all lag days with the largest effect estimate at lag 3; for both CO and NO_2 significant and positive associations were seen at the first 6 lag days, with the largest effect estimate at lag 1. The association between EBC nitrite and O_3 was significant and negative for the first 4 lag days and significant but positive at lag 6. Across the 7 lag days, the largest effect estimates per IQR increase in pollutant concentration were 21.9% for $PM_{2.5}$, 11.1% for SO_4^{2-}, 26.7% for EC, 13.7% for OC, 14.6% for TPN, 20.5% for SO_2, 15.7% for CO, and 22.2% for NO_2. Associations between EBC nitrate and an increase in pollutants varied more substantially across lag days than those of EBC nitrite, both in terms of direction (positive vs. negative) and effect size (see Figure 23). As expected (see Figure 24), the pattern for nitrite+nitrate demonstrated an intermediate response that was in between that of EBC nitrite and EBC nitrate. The largest positive and significant effect estimates were at lag day 0 for $PM_{2.5}$, SO_4^{2-}, EC, and OC, at lag day 1 for TPN and CO, and at lag day 3 for NO_2 and SO_2. The association with O_3 was significant and negative at lag days 0 through 2 and significant and positive at lag day 6.

EBC pH Consistent with the hypothesis that air pollution exposure leads to airway acidification (decreased EBC pH), we observed negative and significant associations of EBC pH with all the pollutants except O_3 at multiple lags (Figure 25). The largest effect estimates were observed at lags 0 and 1 for $PM_{2.5}$, lags 1 and 4 for SO_4^{2-}, lag 0 for EC and OC, lag 1 for TPN, lag 2 for SO_2, lags 0 and 5 for CO, lag 5 for NO_2, and (inversely) lag 0 for O_3.

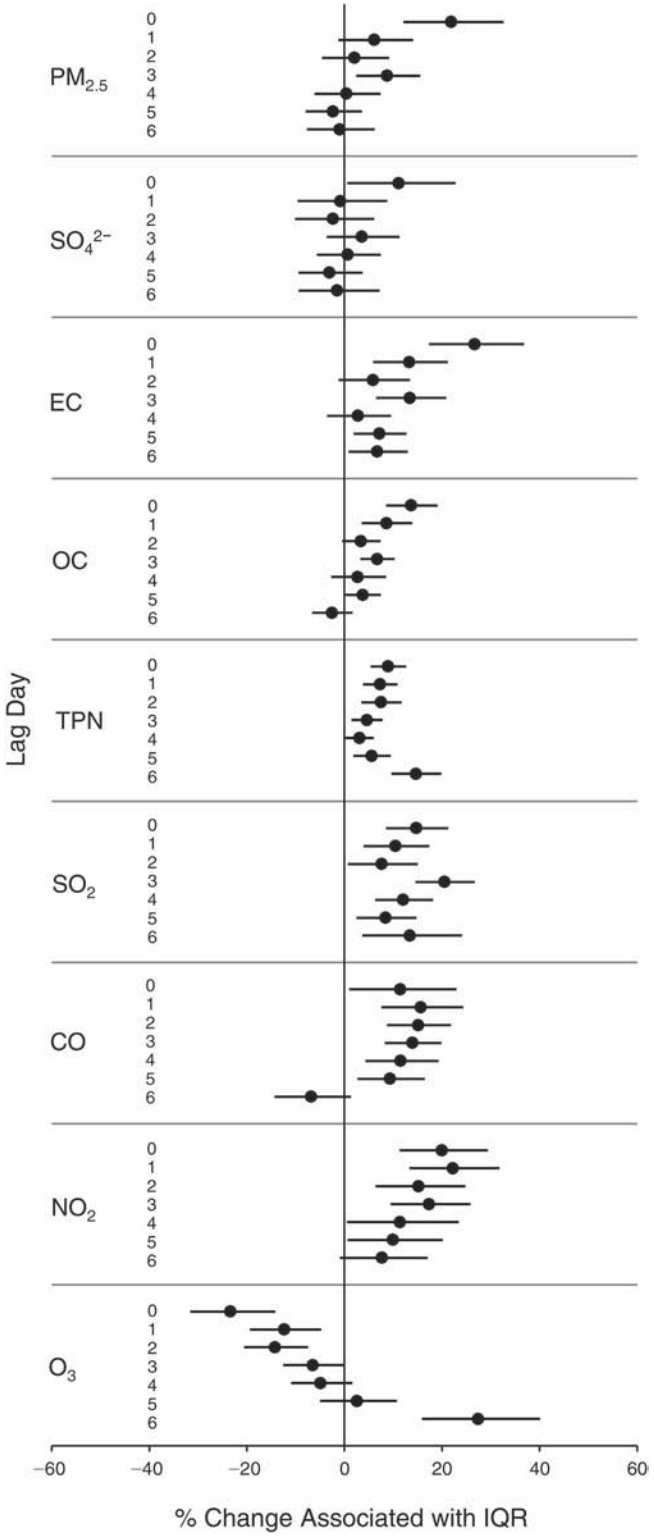

Figure 22. Estimated means and 95% CIs for the percent change in EBC nitrite level associated with one IQR increase in pollutant concentration, controlling for temperature (df in natural spline = 2), RH (df = 1), 7-day moving average of temperature (df = 3), 3-day moving average of RH (df = 3), sex, and day of the week for biomarker measurements.

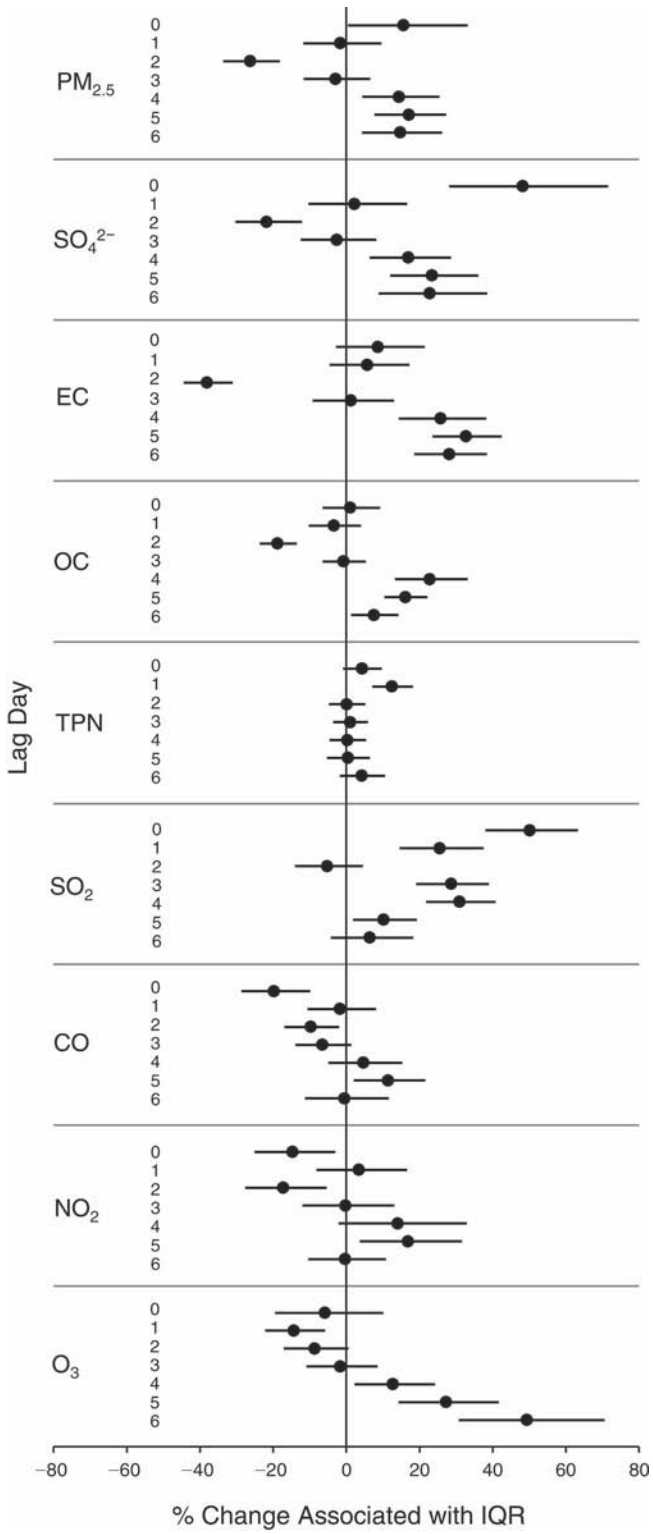

Figure 23. Estimated means and 95% CIs for the percent change in EBC nitrate level associated with one IQR increase in pollutant concentration, controlling for temperature (df in natural spline = 2), RH (df = 1), 7-day moving average of temperature (df = 3), 7-day moving average of RH (df = 3), sex, and day of the week for biomarker measurements.

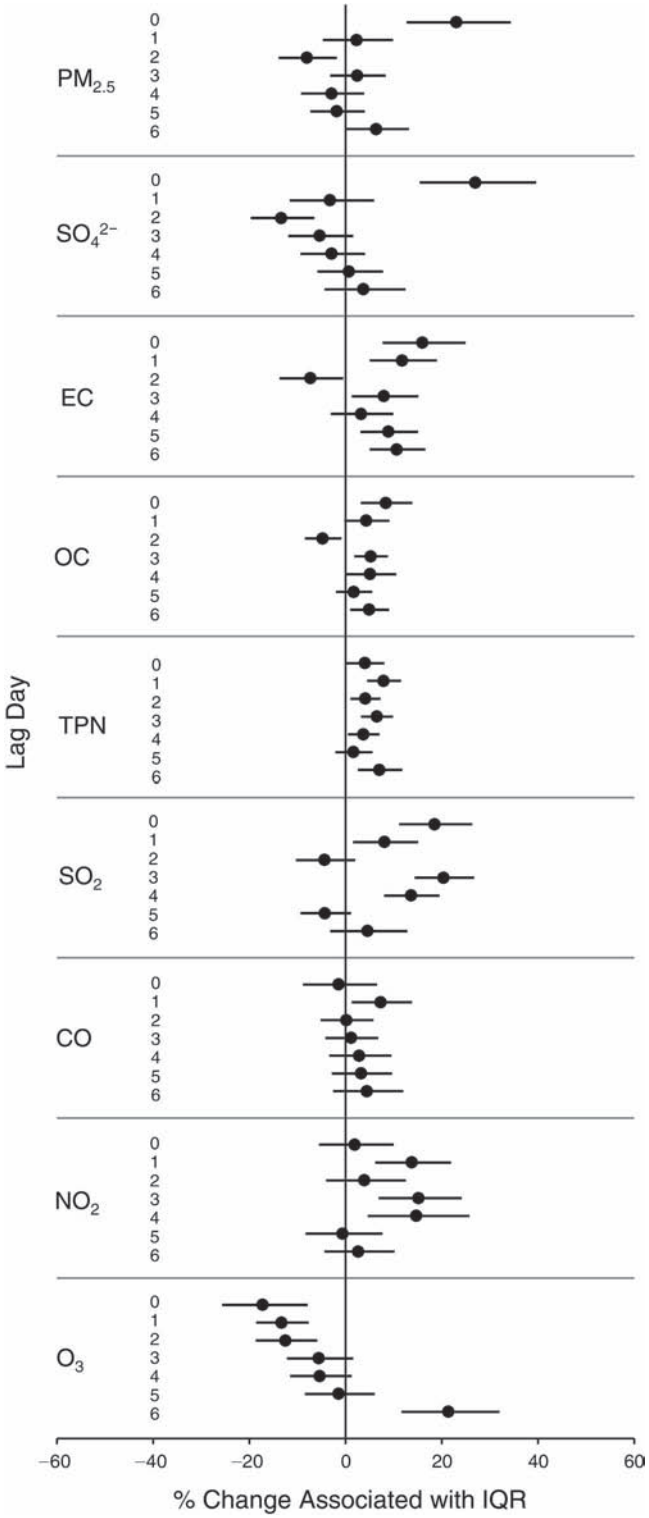

Figure 24. Estimated means and 95% CIs for the percent change in EBC nitrite+nitrate level associated with one IQR increase in pollutant concentration, controlling for temperature (df in natural spline = 1), RH (df = 3), 7-day moving average of temperature (df = 3), 5-day moving average of RH (df = 3), sex, and day of the week for biomarker measurements.

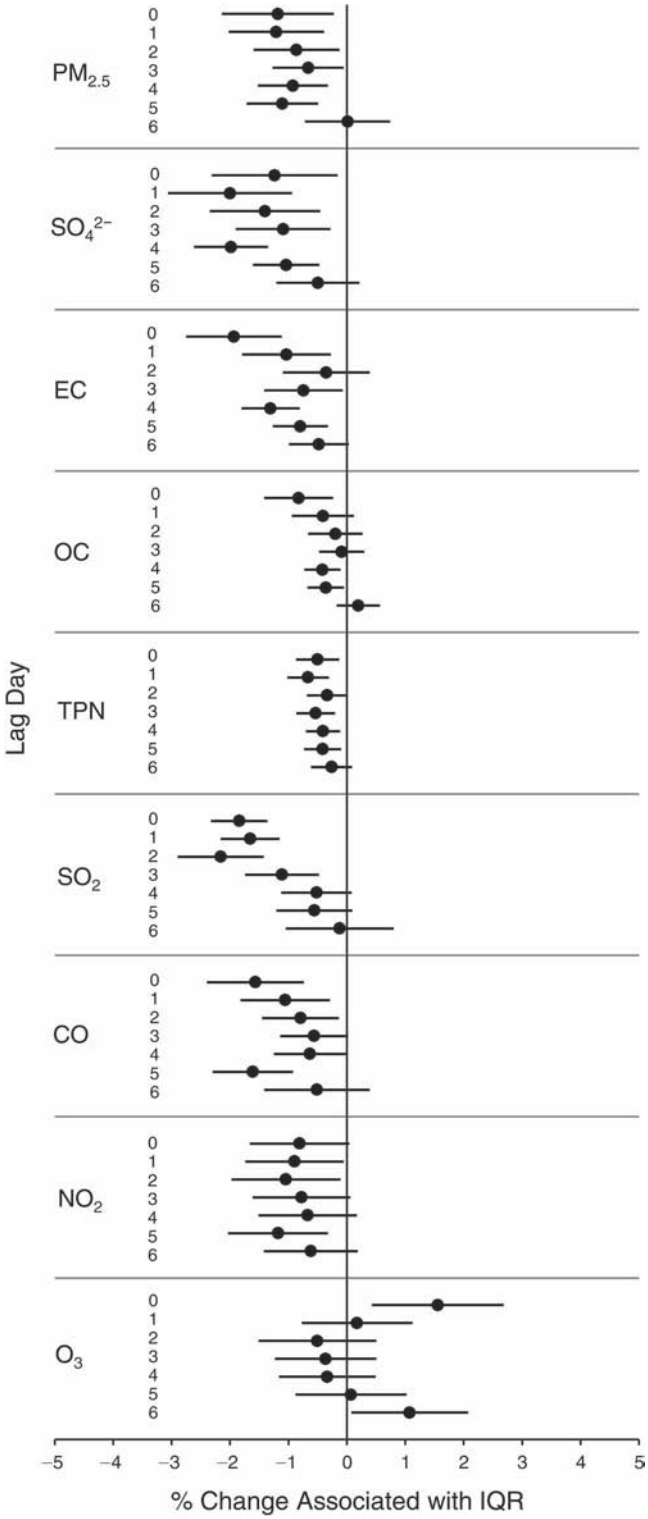

Figure 25. Estimated means and 95% CIs for the percent change in EBC pH level associated with one IQR increase in pollutant concentration, controlling for temperature (df in natural spline = 1), RH (df = 1), 6-day moving average of temperature (df = 3), 3-day moving average of RH (df = 1), sex, and day of the week for biomarker measurements.

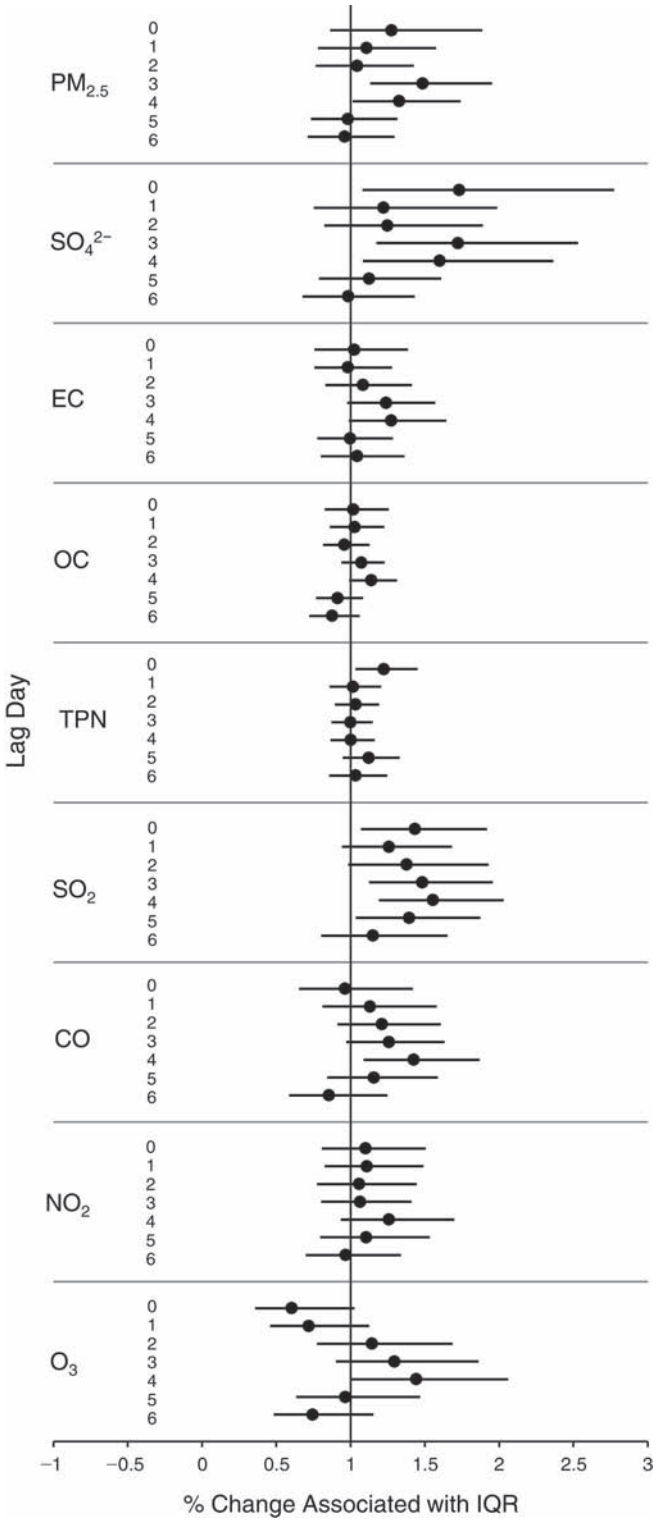

Figure 26. Odds ratios comparing the odds of a greater-than-75th-percentile value of EBC 8-isoprostane to the odds of a smaller-than-75th-percentile value associated with one IQR increase in pollutant concentration, controlling for temperature (df in natural spline = 1), RH (df = 1), 7-day moving average of temperature (df = 2), 2-day moving average of RH (df = 1), sex, and day of the week for biomarker measurements.

EBC 8-Isoprostane Consistent with the hypothesis that air pollution exposure leads to increased oxidative stress in the airways, we observed positive associations between EBC 8-isoprostane (in log odds of having a value >75th percentile) and all the pollutants except O_3 at various lag days (Figure 26). The associations were statistically significant for $PM_{2.5}$ at lags 3 and 4; SO_4^{2-} at lag days 0, 3, and 4; EC at lag day 4; OC at lag 4; TPN at lag day 0; SO_2 at lags 0, 3, 4, and 5; CO at lag 4; and O_3 at lag 4 (positive association) and at lag 0 (negative marginally significant association).

Biomarkers of Hemostasis

sCD62P and sCD40L We observed significant increases in sCD62P associated with IQR increases in all pollutants but O_3, with the largest increases per pollutant (ranging 3.4%–19.1%) at lags 1 to 3 (Figure 27). In contrast, we observed a 12% decrease in sCD62P associated with each IQR increase in O_3 concentration at lag 0. The lag-day association patterns were quite consistent across pollutants. In comparison, sCD40L increases were smaller (ranging 2.9%–7.4%), but still significantly associated with IQR increases in $PM_{2.5}$, SO_4^{2-}, EC, and SO_2 concentrations at lags 3 to 5 (Figure 28).

Platelet Aggregation Contrary to the hypothesis that air pollution leads to increased platelet aggregation, we observed significant decreases in the fraction of platelets aggregated with increases in all pollutants but O_3 at least for the first several lag days (Figure 29). At later lag days, increases in platelet aggregation were associated with increases in concentrations of $PM_{2.5}$, SO_4^{2-}, OC, and SO_2. In contrast, increases in platelet aggregation were significantly associated with increased O_3 concentrations at earlier lag days (0 and 1).

vWF As shown in Figure 30, we observed significant increases (2.5%–8.2%) in vWF associated with IQR increases in most pollutants at various lags, with the largest effects associated with $PM_{2.5}$, SO_4^{2-}, NO_2 and EC (all at lag 3), SO_2 (at lags 1–3), and TPN, OC, and CO (at lags 0 and 1). In contrast, we observed a significant decrease (19.1%) in vWF associated with each IQR increase in O_3 at lag day 0.

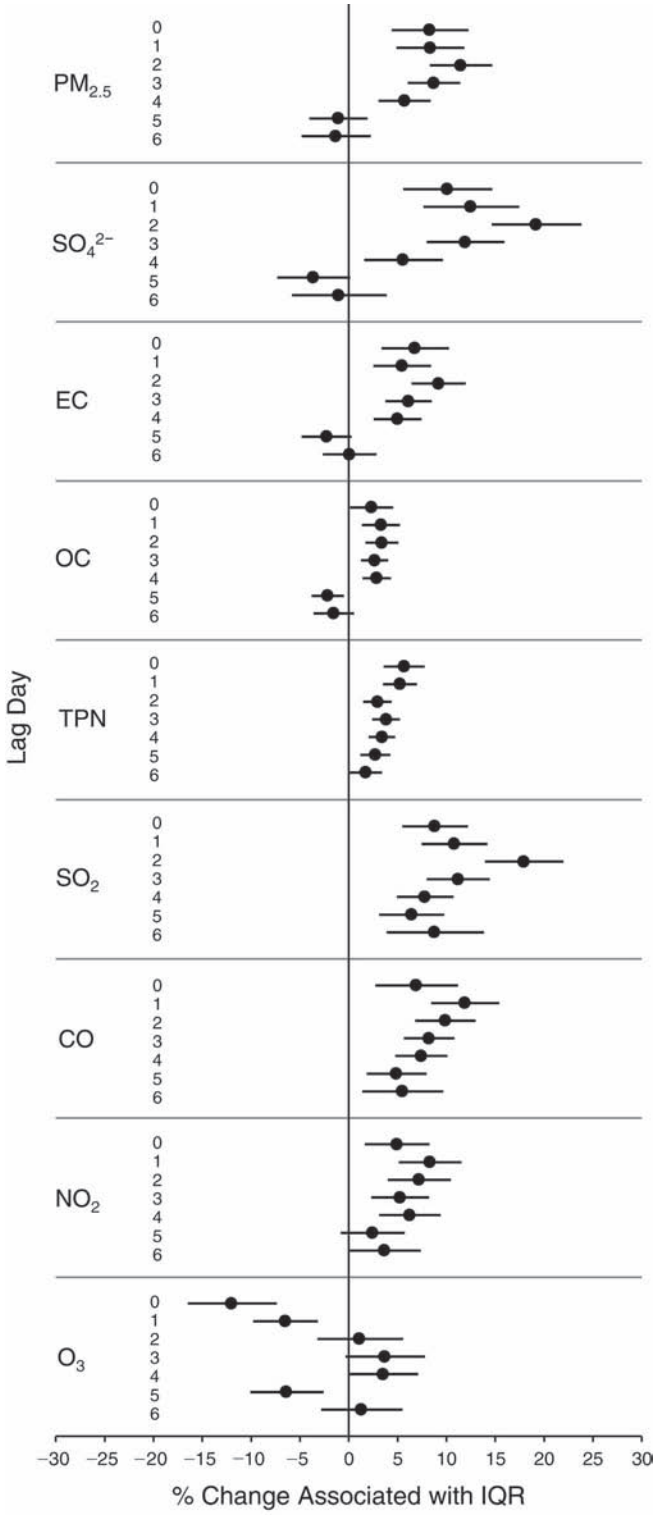

Figure 27. Estimated means and 95% CIs for the percent change in sCD62P level associated with one IQR increase in pollutant concentration, controlling for temperature (df in natural spline = 1), RH (df = 3), 7-day moving average of temperature (df = 2), 4-day moving average of RH (df = 2), sex, and day of the week for biomarker measurements.

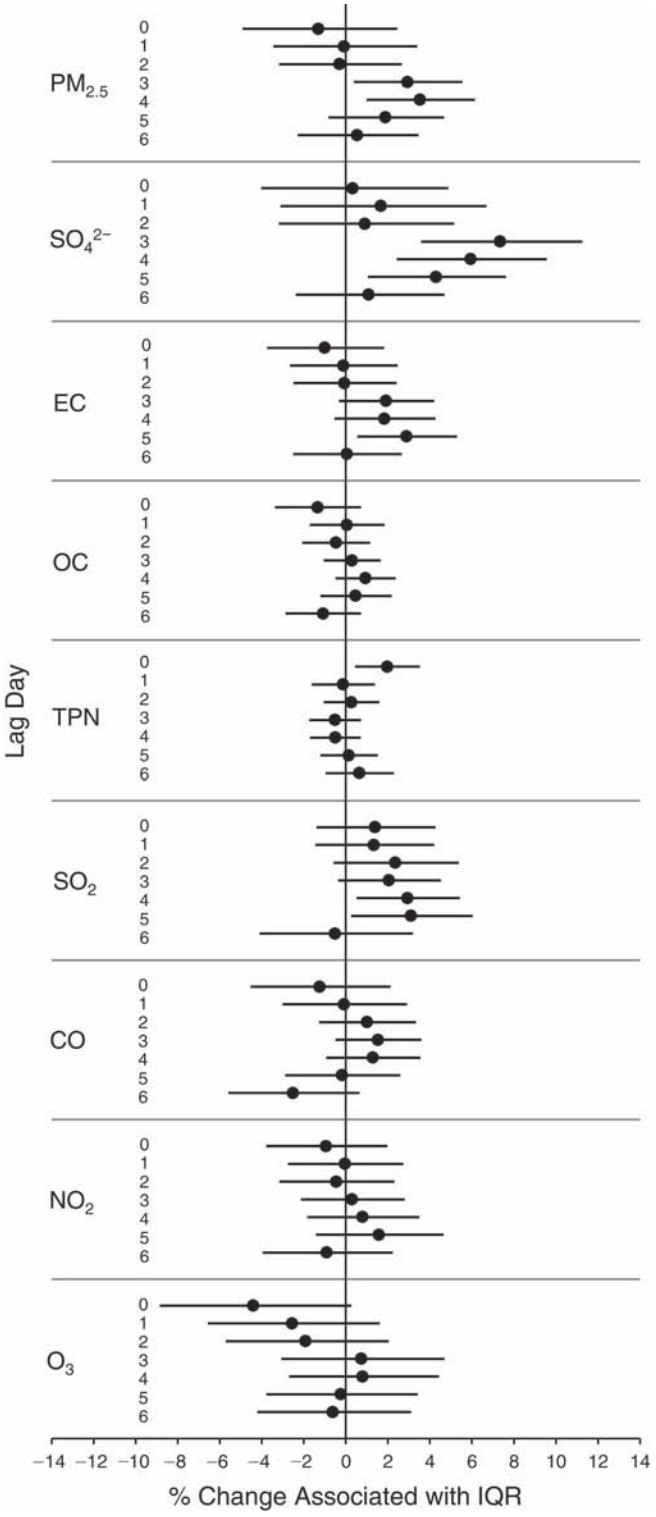

Figure 28. Estimated means and 95% CIs for the percent change in sCD40L level associated with one IQR increase in pollutant concentration, controlling for temperature (df in natural spline = 1), RH (df = 1), 5-day moving average of temperature (df = 1), 2-day moving average of RH (df = 1), sex, and day of the week for biomarker measurements.

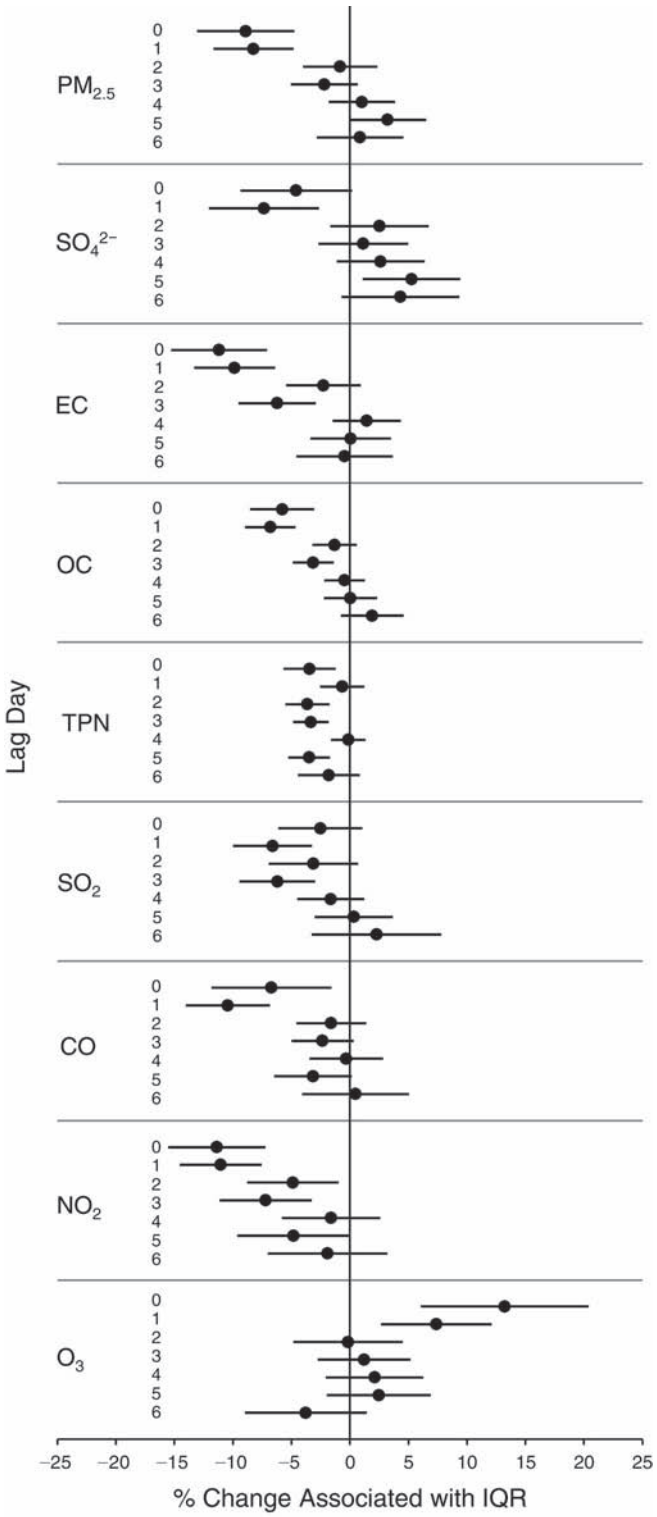

Figure 29. Estimated means and 95% CIs for the percent change in platelet aggregation level associated with one IQR increase in pollutant concentration, controlling for temperature (df in natural spline = 3), RH (df = 3), 7-day moving average of temperature (df = 3), 3-day moving average of RH (df = 3), sex, and day of the week for biomarker measurements.

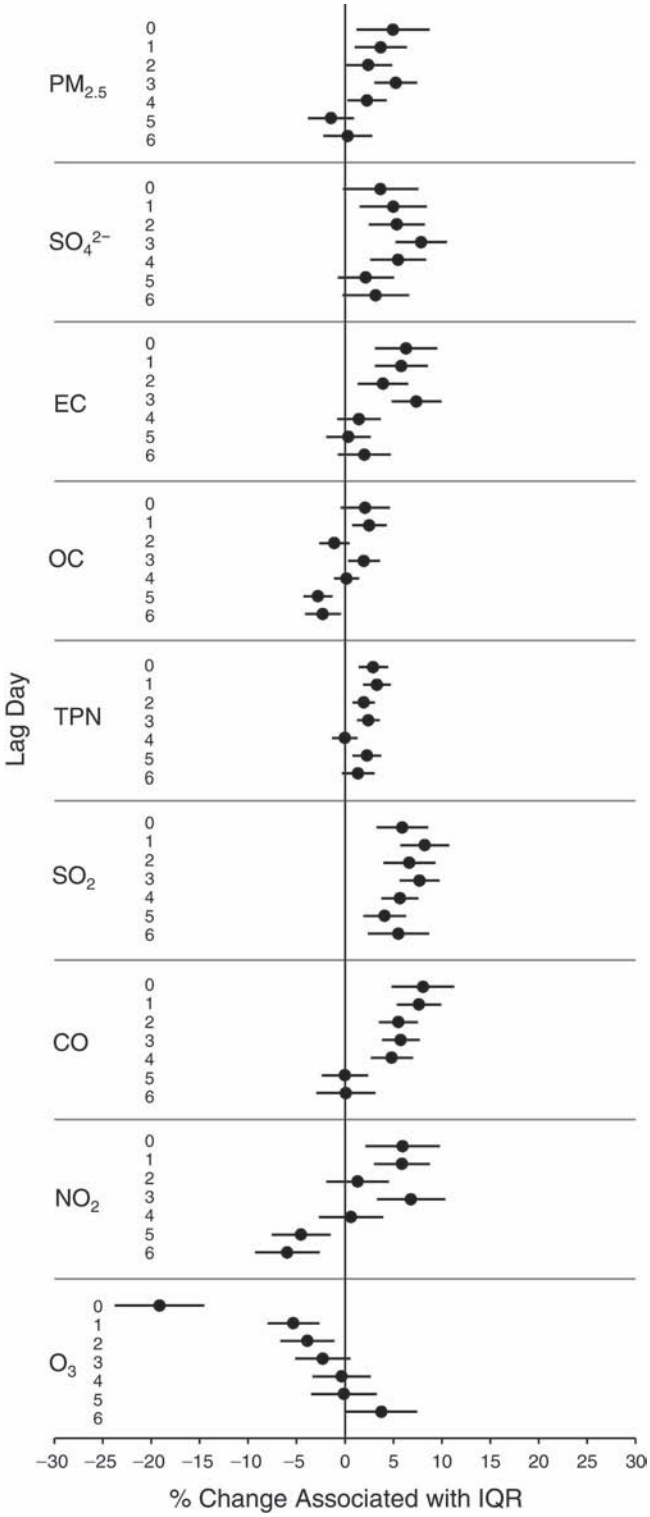

Figure 30. Estimated means and 95% CIs for the percent change in vWF level associated with one IQR increase in pollutant concentration, controlling for temperature (df in natural spline = 3), RH (df = 3), 7-day moving average of temperature (df = 3), 6-day moving average of RH (df = 3), sex, and day of the week for biomarker measurements.

DISCUSSION

AIR QUALITY CHANGES

We observed substantial decreases in air pollutant concentrations (except for O_3) in an unprecedented eight-week air pollution intervention, consistent with measurements previously reported (Wang and Xie 2009; Wang et al. 2009; Zhou et al. 2010; Lin et al. 2011). Mean during-Olympics concentrations were reduced by up to 60% compared with pre-Olympics levels (Figure 6). The largest reductions were for SO_2, CO, and NO_2 measured at our central Beijing monitoring site at which local motor vehicle traffic emissions were expected to be the dominant pollution source. Similarly, the other two pollutants that were expected to have been emitted by local traffic — EC and TPN — were also significantly reduced. We observed slight increases in O_3 (both 24-hour averages and 1-hour maximums) from the pre-Olympics to during-Olympics period. These were likely largely driven by large reductions in NO_2 (and more specifically NO) brought on by the aggressive traffic controls implemented during the Olympics, leading to the well known "titration" of O_3 by NO (Seinfeld and Pandis 1998). In the post-Olympics period, O_3 concentrations declined likely substantially due to limited photochemical activity in the early autumn season in Beijing. Another secondary product of photochemical reactions, SO_4^{2-}, also had the lowest mean concentration during the post-Olympics period. Because $PM_{2.5}$ emissions came from sources other than just traffic, the changes in $PM_{2.5}$ concentrations across the three periods were relatively smaller than the changes in the pollutants more closely related to traffic emissions (see Figure 6).

In the pre-Olympics period, none of the concentrations for CO, NO_2, and SO_2 exceeded health-based standards or guidelines for air pollutants (WHO 2005). On 2 out of 35 pre-Olympics measurement days, 1-hour maximum O_3 exceeded 120 ppb (the 1-hour-based standard set by the U.S. EPA). In large contrast, $PM_{2.5}$ exceeded the 35 $\mu g/m^3$ U.S. EPA 24-hour-based standard on 34 out of 35 days and reached a maximum daily average of 219 $\mu g/m^3$. Even with substantial reductions, during-Olympics concentrations of $PM_{2.5}$ and some of its constituents (e.g., EC and OC) in Beijing were still higher than concentrations observed in Western cities. The high "baseline" concentration (e.g., a pre-Olympics $PM_{2.5}$ mean concentration of 100.9 $\mu g/m^3$), along with large changes across periods (e.g., a 31.5 $\mu g/m^3$ difference between pre- and during-Olympics means for $PM_{2.5}$), may partly explain why we observed significant changes in outcomes (biomarkers) even in young and healthy adults.

Air quality is affected not only by source emissions but also by meteorology. Although the unprecedented source controls resulted in remarkable reductions in mean concentrations, meteorologic conditions unfavorable for pollution dispersion led to high pollutant concentrations on several days within the during-Olympics period (see Tables P.1 and P.2 in Appendix P), as also reported in previous publications (Wang S et al. 2010). The relative contributions of the air pollution control measures and meteorology to air quality changes have been assessed in previous studies (Wang et al. 2009; Wang S et al. 2010; Wang T et al. 2010). This is, however, out of the main scope of this study, as we were principally interested in examining the impact of changes in pollutant concentrations on health endpoints. The day-to-day variability in meteorology may explain the large IQR values for each subperiod and for the whole period (Tables P.1 and P.2). On the other hand, this variability could have contributed to inaccuracy in estimating exposures in our quasi-experimental "A-B-A" design, as clearly not all the days within the during-Olympics period had lower pollutant concentrations than the pre- and post-Olympics days.

In terms of $PM_{2.5}$ compositional changes, we first assessed the largest contributing constituents: SO_4^{2-}, nitrate, NH_4^+, and OM. The pre- and during-Olympics fractions of SO_4^{2-} in $PM_{2.5}$ were similar (27.6% vs. 28.4%), but the post-Olympics fraction was substantially lower (12.0%) (Figure 9). This was expected because SO_4^{2-} is formed through photo-oxidation of SO_2, and the intensity of solar irradiation in the atmosphere during the post-Olympics period (autumn season) was substantially reduced. Although we measured substantially reduced SO_2 concentrations in the center of Beijing, regional sources of SO_2 may have contributed to the increase of SO_4^{2-} measured at the study site. Nitrate fractions, in contrast, were similar in the pre- and post-Olympics period (17.8% vs. 16.1%) but lower in the during-Olympics period (11.5%). This may reflect the substantial reduction in NO_x emissions during the Olympics. Higher fractions of both EC and OM during the post-Olympics period may reflect increased combustion activities in the early autumn season compared with the summer months of the pre- and during-Olympics periods.

We then assessed several categories of "trace-level" constituents that are more biologically reactive than $PM_{2.5}$, EC, OC, and SO_4^{2-}. Transition metals, capable of producing ROS in vitro and in vivo (Bondy et al. 1998; Sørensen et al. 2005), were reduced by 33% from the pre-Olympics fraction of 1.2% to the during-Olympics fraction of 0.8% and then increased to the post-Olympics fraction of 1.9%. PAHs, with a wide variety of toxic effects, were reduced

by 13%, from the pre-Olympics fraction of 0.08% to the during-Olympics fraction of 0.07% and then further decreased to the post-Olympics fraction of 0.04%. This simple analysis of PM compositional changes by period further suggests the complexity of biomarker changes by period, which may reflect changes in overall air quality including PM compositional changes. Future analyses examining the relations between pathway-specific biomarkers and individual PM species or clusters of PM species may generate insights about the relative importance of different PM species in the mechanistic pathways being addressed in this study.

PERIOD COMPARISONS OF BIOMARKERS

Along with substantial reductions in air pollutant concentrations during the Olympics air pollution control period, we observed concomitant statistically significant changes in most, but not all, biomarkers that reflect postulated mechanisms of cardiorespiratory events induced by air pollution (see Table 6 and Figure 10A). Moreover, many of these biomarker changes reverted after the end of the air quality intervention (see Table 6 and Figure 10B). The biomarker changes were mostly in the directions hypothesized for the mechanisms of PM action in relation to air pollution increases or decreases (Kipen et al. 2010) — specifically, that the concentrations of the biomarkers except EBC pH were expected to be lower when air pollution concentrations were lower but the EBC pH level was expected to be higher when air pollution levels were low. In observational studies of air pollution health effects, findings from a "one-direction" intervention (e.g., high levels of pollution becoming low levels) generally increase the certainty of causal relationships compared with those from "pure" correlation analyses. The observed reversibility of biomarker changes in this "two-directional" intervention study (i.e., high levels of pollution going to low and then back to high), therefore, further enhances certainty about the hypothesized mechanisms that may underlie acute cardiorespiratory effects of air pollution, as discussed below.

Autonomic Mechanism

Decreases in HRV, thought to reflect cardiac autonomic imbalance and worsened cardiovascular prognosis (Greiser et al. 2009), have been associated with short-term increases in ambient $PM_{2.5}$, but mostly in the elderly with or without preexisting cardiovascular diseases (Sullivan et al. 2005; de Hartog et al. 2009; Folino et al. 2009; Zanobetti et al. 2010). Studies of young participants found somewhat inconsistent results regarding the relation between HRV and $PM_{2.5}$, in terms of which HRV indices showed statistically significant effects over varying periods (i.e., lag times) of $PM_{2.5}$ measurements (Vallejo et al. 2006; Chuang et al. 2007; Wu et al. 2009; Lin et al. 2011). Our analysis did not find statistically significant changes in HRV indices from one period to another (Figure 10), despite significant and substantial changes over the three periods in air quality and in the many other biomarkers shown in the figure.

A number of previous studies reported statistically significant positive associations between ambient air pollution and BP (mostly SBP) in the elderly with or without cardiorespiratory disease (Mar et al. 2005b; Bartoli et al. 2009). In young and healthy adults, we observed a marginally significant decrease (~1.6 mmHg) in SBP during the Olympics air pollution control period compared with the pre-Olympics period, and a significant increase (~10 mmHg) in SBP from the during- to the post-Olympics period (see Table 6). We also observed a small but significant decrease (~1 bpm) in HR from the pre- to the during-Olympics period (but a nonsignificant and near-zero change from the during- to the post-Olympics period), somewhat in agreement with the positive HR–PM associations observed in two previous studies (Magari et al. 2002; Schneider et al. 2010). When the positive findings on SBP and partially positive findings on HR but null findings on HRV are considered all together, our period comparisons provide limited evidence to support the hypothesis that air pollution leads to autonomic dysfunction in young and healthy adults.

Inflammatory Mechanism

The most prominent hypothesis on the mechanism relating air pollution to cardiorespiratory mortality and morbidity has been that air pollutants, especially fine and ultrafine particles, provoke pulmonary inflammation, largely by induction of oxidative stress. This oxidative stress then may lead to increased blood coagulability and systemic inflammation over a matter of hours to days (Seaton et al. 1995, 1999). Numerous epidemiologic and experimental studies have demonstrated increased levels of pulmonary inflammation (e.g., indicated by FeNO and other inflammatory markers in airway fluids) associated with air pollution exposure (Ghio et al. 2000; Nemmar et al. 2003a; Koenig et al. 2005; Adar et al. 2007). Epidemiologic studies have also demonstrated associations between air pollution and clinical markers of systemic inflammation including increased WBCs, fibrinogen, vWF, and CRP (Peters et al. 1997; Danesh et al. 1998; Seaton et al. 1999; Pekkanen et al. 2000; Peters et al. 2001a, 2001b; van Eeden et al. 2001; van Eeden and Hogg 2002; Shishehbor et al. 2003; Riediker et al. 2004; Ruckerl et al. 2006; Zuurbier

et al. 2011). Despite some initial positive human studies (Jansen et al. 2005), acute experimental data are not as consistent in demonstrating a clear relationship between particulate air pollution exposure and systemic inflammatory markers (Ghio et al. 2000; Beckett et al. 2005).

An increase in FeNO, a sensitive biomarker of airway inflammation, has been associated with increases in air pollution in asthmatics and children as well as healthy adults (Nightingale et al. 1999; van Amsterdam et al. 1999; Steerenberg et al. 2001; Koenig et al. 2003; Koenig et al. 2005; Mar et al. 2005a; Delfino et al. 2006). In healthy young adults, we observed a statistically significant decrease of 60.3% in FeNO associated with the air quality improvement (i.e., from the pre-Olympics period to the during-Olympics period) and then a statistically significant increase of 130% associated with the relaxation of the pollution control (i.e., from the during-Olympics to the post-Olympics period).

Our study is perhaps the first in which EBC pH was measured in healthy young adults. Studies of diseased adults or the elderly have reported that decreases in airway pH causes bronchoconstriction and impairs ciliary motility (Ricciardolo et al. 1999), increases airway mucus viscosity (Holma 1989), and induces damage to the airway epithelium (Holma et al. 1977). One panel study that measured EBC pH in asthmatic patients suggested that acute asthma exacerbations may be accompanied by airway acidification that reflects the inhibition of local epithelial proton pumps during airway inflammation (Antus et al. 2010). Statistically significant decreases in EBC pH were reportedly associated with exposure to diesel traffic in adults with asthma (McCreanor et al. 2007) and with exposure to O_3 in children with asthma (Barraza-Villarreal et al. 2008). However, the limited research on the association of EBC pH with ambient pollution has generated inconsistent findings (McCreanor et al. 2007; Epton et al. 2008; Ferdinands et al. 2008; Romieu et al. 2008). In this study, we observed a statistically significant increase in EBC pH (exhibited by a 46% decrease in EBC hydrogen ion concentration) associated with the air pollution intervention and a significant increase (146%) in EBC hydrogen ion concentration associated with the relaxation of the pollution controls.

Our findings in pulmonary inflammatory markers are consistent with the hypothesis that inhaled PM induces the release of pro-inflammatory mediators leading to a local inflammatory response (Danesh et al. 2000). Inflammatory mediators can leave the lung and enter the circulation, inducing systemic effects (van Eeden et al. 2001; van Eeden and Hogg 2002; Shishehbor et al. 2003). In the liver, these mediators initiate an acute phase response, characterized by increased production of acute phase proteins, including fibrinogen and CRP, which previous studies have suggested are associated with exposure to PM (Zanobetti et al. 2000; Atkinson et al. 2001; Peters et al. 2001a). In the present study, we found no change in plasma fibrinogen level from the pre- to the during-Olympics period but a marginally significant increase of 4.3% from the during- to post-Olympics period (Figure 10). A crude analysis of our CRP data (with a large fraction of below-detection values due to the low sensitivity of the assay) found that about 10% more samples (data not shown) were below detection in the during-Olympics period compared with the pre-Olympics period, suggesting improved air quality was associated with lower concentrations of CRP.

Elevation of WBC count even within the normal range is a marker for increased cardiovascular disease risk. For each 1.0×10^9/L increment in WBCs, cardiovascular disease risk increased by 32% in nonsmokers (Seaton et al. 1995). WBC increases have also been shown to occur acutely following exposure to fresh diesel exhaust (Seaton et al. 1999). In the present study, however, we observed small and nonsignificant increases in WBCs from the pre-Olympics period to the during-Olympics period and small and nonsignificant decreases in WBC from the during-Olympics to the post-Olympics periods (see Figure 10). Our findings on the directions of WBC changes in relation to the changes in overall air quality appear to be contrary to the hypothesis. However, the changes in this biomarker were not statistically significant and were relatively small. Our findings were in agreement with the null results (nonsignificant effects) from two short-term, experimental (chamber) studies with concentrated ambient particles or fresh diesel exhaust (Ghio et al. 2000, 2003) but were in contrast to another chamber study that showed that diesel exhaust exposure led to increased WBC counts (Salvi et al. 1999). In addition, our findings were in contrast to two observational studies that showed significant associations between WBC counts and air pollution (Seaton et al. 1999; Ruckerl et al. 2007).

We observed a marginally significant increase in lymphocyte counts from the pre-Olympics to the during-Olympics period and a significant reverse change from the during- to the post-Olympics period. As the change in lymphocyte counts associated with the change in air pollution exposure has not been previously reported, its biologic relevance cannot be readily determined.

Hemostasis Mechanism

Increased blood coagulation is another prominently hypothesized mechanism to explain the increased cardiorespiratory (especially cardiovascular) morbidity and

mortality associated with air pollution (Brook et al. 2010). Platelet activation leading to thrombosis is now widely recognized to underlie acute complications of atherosclerosis such as unstable angina and myocardial infarction (Davi and Patrono 2007). While inflammation is acknowledged to be a key pathophysiologic mechanism for the initiation of acute thrombosis, direct activation of platelets by exposure to particulate air pollution may also occur (Trenga et al. 2006). Platelets normally circulate in the blood for 3 to 4 days in a resting state and form thrombi only after they are activated by exposure to an agonist such as the lipid core of arterial plaques or alpha-thrombin, or to some type of environmental stimuli such as exercise (Gold et al. 2000) or apneic sleep (Creason et al. 2001). Limited research has suggested that air pollution exposure (especially to PM) leads to increased platelet activation and platelet aggregation. For example, rodent and in vitro studies have demonstrated rapid platelet aggregation and thrombosis with intratracheal instillation of various ultrafine particles (Nemmar et al. 2002, 2003a, 2003b; Radomski et al. 2005). One observational study in older diabetics found a significant association between acute PM exposure and an increase in ex vivo measurement of platelet activation (Jacobs et al. 2010), while an experimental study with laboratory-controlled diesel exposure showed an increase in thrombus formation (Lucking et al. 2008). Positive associations between sCD62P and PM exposure have been reported in one observational study (Delfino et al. 2009) and one experimental study (Stewart et al. 2010), and sCD40L has been reported to increase in association with PM exposure (Ruckerl et al. 2006).

In the present study, we found a significant 34% reduction in sCD62P and a significant 5.7% reduction in sCD40L in the during-Olympics period compared with levels in the pre-Olympics period. Further, we found a significant 33.7% increase in sCD62P and a significant 9.1% increase in sCD40L from the during-Olympics to the post-Olympics period. Our findings on these two biomarkers, consistent with the previous findings described above, support the hypothesis that platelet activation is an important mechanism in mediating acute air pollution effects on cardiovascular risk and provide an attractive explanation for the previously reported triggering of myocardial infarction by exposure to ambient PM (Peters et al. 2001b, 2004; D'Ippoliti et al. 2003; Zanobetti and Schwartz 2005; Pope et al. 2006; Rich et al. 2010).

Elevated plasma vWF levels, reflecting endothelial dysfunction, have been linked to hemostasis as well as systemic inflammation (Zezos et al. 2005). Levels of vWF in the circulation were reported to increase 24 hours after exposure onset in an occupational study of police officers (Symons et al. 2006), although a controlled study reported no change in vWF after 1 hour of exposure to 300 $\mu g/m^3$ diesel exhaust (Rich et al. 2004). In the present study, we found a significant decrease of 13% in vWF level from the pre- to the during-Olympics period but did not see a reverse change (increase) in this biomarker from the during- to the post-Olympics period. In contrast, for platelet aggregation, we found a significant 7.4% increase from the pre- to the during-Olympics period and a significant 41% reverse change (decrease) from the during-Olympics period to the post-Olympics period. The soluble platelet activation and platelet aggregation marker results appear to be in conflict, findings that will be discussed later in the section "Pollutant-Specific Effects."

Oxidative Stress Mechanism

Much of the effort to identify a biochemical basis to explain both the acute and chronic effects of air pollution has been focused on the role of ROS (e.g., free radicals and peroxides) induced from exposure to air pollution, especially to certain constituents of PM (Nel 2005). The presence of ROS may lead to the generation of oxidative stress, and then proinflammatory effects, both local to (e.g., in the respiratory tract) and distant from (e.g., in the systemic circulation) the site of injury (Gilliland et al. 1999; Becker et al. 2005; Donaldson et al. 2005; Nel 2005).

Both EBC nitrite and EBC nitrate (or the sum of nitrite and nitrate) are products of the metabolic oxidation of NO, produced primarily in the lung by inducible nitric oxide synthase, reflecting levels of pulmonary oxidative and nitrosative stress (Hunt et al. 2000; Kostikas et al. 2002). ROS can react with lipids to form stable compounds such as 8-isoprostane. Increased levels of EBC nitrite and/or nitrate and 8-isoprostane have been associated with asthma, chronic obstructive pulmonary disease, and cystic fibrosis (Corradi et al. 2003b; Kostikas et al. 2003; Robroeks et al. 2007; Barreto et al. 2009; Rihak et al. 2010). However, to the best of our knowledge, there have been no published studies reporting EBC nitrite and/or nitrate in relation to air pollution exposure and only one study reporting increases in EBC 8-isoprostane concentration associated with air pollution exposure in children with asthma (Liu et al. 2009).

In healthy adults, we found statistically significant reductions ranging from 17.6% to 30% in EBC nitrite, nitrate, and nitrate+nitrite during the Olympics air pollution intervention period compared with pre-Olympics levels (Figure 10). We also observed large and significant reverse changes (increases of 124% to 161%) for all three of these biomarkers in the post-Olympics period compared with during-Olympics levels. These changes are not likely

due to changes in room NO_x concentrations, based on a simple calculation, as follows. Even if all the NO_2 molecules in the ambient air were absorbed in the airway when they were inhaled by the subject and were converted to nitrite and nitrate, the steady-state EBC concentration of nitrite+nitrate resulting from this source would account for only <0.1% of the nitrite+nitrate concentrations measured in the study. The fraction of above-detection samples for EBC 8-isoprostane was the lowest (44%) during the Olympics period compared with 68% in the pre-Olympics period and 74% in the post-Olympics period (Table 6). Our findings on all these EBC biomarkers support the hypothesis that air pollution exposure leads to an increased burden of oxidative stress in the respiratory tract.

ROS can oxidize DNA molecules to form stable products such as 8-OHdG. Increased 8-OHdG levels have been positively associated with premature mortality due to coronary heart disease (Collins et al. 1998) but negatively associated with total serum antioxidant capacity (Vassalle et al. 2004; Demirbag et al. 2005). Measurement of 8-OHdG in urine has been used to assess whole-body oxidative DNA damage and has been suggested by the National Institute of Environmental Health Sciences's Biomarkers of Oxidative Stress Study to be a useful biomarker of systemic oxidative stress (Kadiiska et al. 2005). Previous studies have demonstrated an association between urinary 8-OHdG and exposure to ambient PM, especially ROS-inducing constituents of PM (e.g., transition metals and PAHs) (Ren et al. 2010; Wei et al. 2010). In the present study, we observed a large (58%) and statistically significant reduction in urinary 8-OHdG concentration from the pre- to the during-Olympics period and a very large (315%) reverse change (increase) from the during- to the post-Olympics period (Figure 10). This finding supports an association between lowered whole-body oxidative stress and improved air quality.

Although there is a considerable literature reporting associations between exposure to ambient PM and oxidative stress in children and adults with asthma or other cardiorespiratory diseases (Baraldi et al. 2003; Corradi et al. 2003a; Koenig et al. 2003; Adamkiewicz et al. 2004; Romieu et al. 2008), this study is the first to provide multi-biomarker evidence for PM acting through oxidative stress mechanisms in young and healthy adults both in the respiratory tract and systemically.

POLLUTANT-SPECIFIC EFFECTS

The findings from our "A-B-A" design, as discussed earlier, may reflect the effects of air pollution as a whole mixture, rather than the action of one or more isolated individual pollutants. It is also possible that the observed biomarker changes by period were due to factors other than air pollution. Hence, our panel analysis results on pollutant–biomarker relationships provide complementary and confirmatory evidence that the biomarker changes across the three periods were most likely attributable to air quality changes. We indeed observed a remarkable consistency in findings between the pollutant–biomarker association panel analysis and the period analysis, although this is not surprising from a statistical analysis perspective, because the period analysis and the pollutant–biomarker analysis were not independent (i.e., differences in pollutant concentrations between periods were correlated with daily pollutant concentrations). The biomarkers that showed large and significant period effects also showed strong and significant associations with all or most of the pollutants for multiple lag days. These included all the respiratory biomarkers (FeNO, EBC pH, nitrate, nitrite, and nitrite+nitrate), urinary 8-OHdG, both platelet activation markers (sCD62P and sCD40L), vWF, and platelet aggregation. When the period analysis showed a small and/or nonsignificant change for a particular biomarker, the pollutant–biomarker associations were usually either small or inconsistent across pollutants. For example, HRV indices did not change across periods and were associated with only a few pollutants (e.g., LF with $PM_{2.5}$ only [data not shown]; HF with SO_4^{2-} and NO_2 [data not shown]; VLF with EC and SO_4^{2-}; and SDNN with $PM_{2.5}$, SO_4^{2-}, OC, and NO_2) at a few lag days. For those biomarkers that showed significant associations with some of the measured pollutants, a more in-depth analysis of PM composition and/or pollution sources may generate additional insights about source-specific or composition-specific effects. This may be done in future analyses of the PM composition data collected in this study (Appendix A), perhaps along with additional data on source profiles.

It has been of great interest, both from a scientific standpoint and from a regulatory perspective, which components of the air pollution mixture (e.g., gases vs. PM) and which constituents of PM are more toxic than others. We attempted to address this question through our pollutant–biomarker association analysis, but correlations among pollutants, due in part to the simultaneous shutting down of multiple pollutant sources during the Olympics and the subsequent relaxation of pollution controls, made it difficult to differentiate effects of specific pollutants. In particular, although we found statistically significant associations for most of the pollutants, including O_3, with specific biomarkers, the associations with O_3 were typically in the opposite direction. The seemingly "beneficial" effect of O_3 on several biomarkers is likely due to the fact that O_3 concentrations were increased while the other pollutants were

substantially reduced during the Olympics air pollution intervention period and that O_3 was negatively correlated with NO_2 and other pollutants (e.g., CO, TPN, and EC). A similar, seemingly protective effect of O_3 has also been observed in previous studies (Anderson et al. 1998; Hajat et al. 1999) probably for the same reason.

Potential Confounding from Copollutants

Given that many of the pollutants were correlated (see Table 5), we examined whether significant pollutant–biomarker associations from the single-pollutant models were independent of other pollutants. We ran two-pollutant models by including the second pollutant concentration at the lag of maximum effect from the significant single-pollutant model finding. We plotted the results, as shown in Appendix C. When adjusting for a second pollutant in the same model, we observed small changes in effect estimates for most of the pollutant–biomarker pairs, although the adjustment resulted in the loss of statistical significance for most of the pollutant–biomarker relationships. As shown in Appendix C, adjusting for a second pollutant did not change the statistical significance of the $PM_{2.5}$ effects for the following five biomarkers: RBC, 8-OHdG, FeNO, nitrate, and sCD62P. This adjustment also left SO_4^{2-} effects statistically significant for the following eight biomarkers: 8-OHdG, FeNO, EBC pH, 8-isoprostane, sCD62P, sCD40L, vWF, and SDNN. In contrast, adjusting for a second pollutant resulted in the loss of statistical significance for EC and OC effects for most biomarkers; EC effects remained significant only for RBC, EBC nitrate, and sCD62P, while OC effects remained significant only for vWF. After adjusting for a second pollutant, the TPN effects remained significant for the following six biomarkers: WBCs, 8-OHdG, FeNO, EBC nitrite+nitrate, sCD62P, and vWF. Among the gaseous pollutants, the second-pollutant adjustment had the largest impact on the statistical significance of NO_2 effects; however, only the effects of NO_2 on RBC remained significant. In contrast, the statistical significance of the SO_2 effects was sustained for the following nine biomarkers: LF/HF ratio, fibrinogen, lymphocytes, EBC nitrite, EBC nitrate, nitrite+nitrate, EBC pH, sCD62P, and vWF. The CO effects remained statistically significant for the following three biomarkers: FeNO, sCD62P, and platelet aggregation. Interestingly, after adjusting for a second pollutant, the adverse effects of O_3 (shown as a positive association between the pollutant and biomarker) remained statistically significant for FeNO, EBC nitrite, nitrate, and nitrite+nitrate (all biomarkers of pulmonary inflammation and oxidative stress), while the seemingly "protective" effects remained statistically significant for three cardiovascular-related biomarkers (SBP, sCD62P, and vWF). Although small changes were observed in the overall pattern of the associations and in the effect estimates, the loss of statistical significance by adjusting copollutants was substantial. It is intriguing to speculate on the reasons for these findings, and any interpretation needs to be augmented with additional insights from further data analyses (e.g., analyses relating source-specific pollutants or specific $PM_{2.5}$ species, such as transition metals and PAHs, to specific biomarkers).

For the six biomarkers 8-OHdG, sCD62P, sCD40L, FeNO, EBC nitrite, and vWF, alternative two-pollutant models in which the lag for both pollutants was the same were compared with the two-pollutant models discussed above. The results are shown in Appendix F. We observed little or no difference in the results from these two different two-pollutant models.

Associations Contrary to Hypotheses

For a few biomarkers, we observed statistically significant associations with pollutants but in the direction opposite to that hypothesized. These include RBC, WBC, and platelet aggregation, each of which was negatively associated on multiple lag days with most of the pollutants except O_3 (see Figures 17, 18, and 29, respectively). As discussed earlier, previous findings on associations between WBC or RBC with air pollution exposure are inconsistent (Seaton et al. 1999; Ghio et al. 2000, 2003; Ruckerl et al. 2007; Lucking et al. 2008), and our results add further complexity to this limited literature and may suggest that mechanisms other than inflammation and hemostasis are involved in the response to air pollution. For example, one controlled human exposure study has shown concentration–response reductions in blood hemoglobin in response to NO_2 exposure with intermittent exercise (Frampton et al. 2002). High concentrations of NO_2 are known to cause hemolysis (Frampton et al. 2002), and the negative association between RBC count and NO_2 observed in the present study was particularly large in effect estimates, strong on statistical significance, and consistent across lag days (Figure 17). This finding, along with previous findings (Seaton et al. 1999; Frampton et al. 2002), suggests hemolysis as a potential mechanism by which air pollution may exert cardiovascular effects. Since this mechanism was not among the ones this study aimed to examine, we did not collect blood specimens that would allow us to investigate this further. Future studies of hemolysis as a possible mechanism underlying cardiovascular effects of air pollution are warranted.

Strikingly, our findings on the two soluble platelet activation markers (sCD40L and sCD62P) and on platelet aggregation appear to be in conflict. One experimental study (which measured both thrombosis and sCD62P)

and one observational study demonstrated a rapid tendency toward increased coagulation following exposure to increased concentrations of PM (Lucking et al. 2008; Jacobs et al. 2010). Thus, some kind of compensatory mechanism to explain the contradiction does not seem likely. In our study, the aggregation with epinephrine was not done using the concentration of epinephrine most recently recommended for use in studies seeking hyperreactive (procoagulant) rather than bleeding tendency outcomes (Yee et al. 2005). However, we do not think this methodologic discrepancy is likely to lead to the paradoxical result we report in this study. We explored the possibility of pollutant-mediated platelet activation leading to fewer (inactivated) platelets available for aggregation (i.e., more prior platelet-activation aggregation leading to fewer resting platelets available to be aggregated). We re-ran our analytic models for $PM_{2.5}$ and platelet aggregation with and without adjustment for sCD62P and sCD40L, but these adjustments made no appreciable difference in the results, not lending support to this explanation. We also explored whether there was increased platelet aggregation associated with pollutant concentrations in the few hours before the clinic visits for biomarker measurements (i.e., lag 0–2 or 0–5 hours), with compensatory aggregation reductions in the later hours in the first day (i.e., lags 6–23 hour), leading to an overall decreased aggregation response to pollution on lag day 0 (i.e., lags 0–23 hour). To assess this, we considered whether our 24-hour averages of pollutants might miss possible rapid effects on platelet aggregation that occurred over a few hours. Analyses of hourly pollution data, however, did not support this as an explanation, as there were no increased platelet aggregation effects observed for any 3-hour block of $PM_{2.5}$ concentrations.

Table 8. Pollutant-Biomarker Associations That Had Largest Effect Estimates, Were Statistically Significant, and Moved in the Hypothesized Direction at Specified Lag Days

Pollutant / Lag Day	Biomarker
$PM_{2.5}$	
0	FeNO, EBC pH, EBC nitrite, EBC nitrite+nitrate
1	Heart rate, 8-OHdG
2	Fibrinogen, sCD62P
3	SBP, 8-isoprostane, vWF
4	sCD40L
5	EBC nitrate
6	—
SO_4^{2-}	
0	FeNO, EBC nitrite, EBC nitrite+nitrate, 8-isoprostane
1	EBC pH, 8-OHdG, Heart rate
2	sCD62P
3	vWF, sCD40L
4	—
5	—
6	—
EC	
0	FeNO, EBC pH, EBC nitrite, EBC nitrite+nitrate
1	8-OHdG
2	Fibrinogen, sCD62P
3	SBP, vWF, Heart Rate
4	sCD40L
5	EBC nitrate
6	—
OC	
0	EBC pH, EBC nitrite, EBC nitrite+nitrate, SBP
1	vWF, 8-OHdG, sCD62P
2	Fibrinogen, sCD62P
3	Fibrinogen
4	FeNO, EBC nitrate
5	—
6	—

(*Table continues on next page*)

LAG PATTERNS AND TIMING OF ACTIONS

The present study focused on the acute effects of air pollution exposure. However, the meaning of "acute" (e.g., within hours, a day, or several days) is imprecise for any of the mechanistic pathways addressed in the present study. We used 24-hour average concentrations, measured from 1 to 7 days before biomarker measurement (lag days 0 to 6, respectively). (Because we used the real-time monitor to measure the gaseous pollutants and $PM_{2.5}$, resulting in hourly measurement data, it is possible to evaluate their effects within a shorter time frame if such analyses are deemed necessary in the future.)

To help gain an overall picture about the timing of effects, we simplified most of the information presented in the figures and summarized it in Table 8. In the table, we just showed the lag day, among all the lag days, at which a pollutant was significantly associated with a biomarker in the hypothesized direction and had the largest effect estimate. As shown in Table 8, most of the biomarkers of pulmonary inflammation and oxidative stress (FeNO, EBC pH, EBC nitrite, and EBC nitrite+nitrate) showed the largest effect at early lag days (lag 0 and 1) for most of the

Table 8 (*Continued*). Pollutant-Biomarker Associations That Had Largest Effect Estimates, Were Statistically Significant, and Moved in the Hypothesized Direction at Specified Lag Days

Pollutant / Lag Day	Biomarker
TPN	
0	FeNO, 8-isoprostane, sCD40L, sCD62P
1	EBC nitrate, EBC nitrite+nitrate, EBC pH, vWF
2	—
3	8-OHdG, Heart rate
4	SBP
5	—
6	EBC nitrite
SO_2	
0	EBC nitrate
1	vWF, 8-OHdG, Heart rate
2	FeNO, sCD62P, EBC pH
3	EBC nitrite, EBC nitrite+nitrate, SBP
4	8-isoprostane
5	sCD40L
6	Fibrinogen
CO	
0	FeNO, vWF
1	EBC nitrite, EBC nitrite+nitrate, sCD62P, SBP, 8-OHdG
2	—
3	—
4	8-isoprostane
5	EBC pH, EBC nitrate
6	—
NO_2	
0	FeNO
1	EBC nitrite, sCD62P, 8-OHdG, EBC nitrite+nitrate
2	—
3	SBP, vWF
4	—
5	EBC pH, EBC nitrate
6	—

pollutants. EBC nitrate, in contrast, usually showed the largest effect at later lag days (lags 4 or 5). The difference in the lag-day effects between EBC nitrite and nitrate suggests that the change from nitrite to nitrate in the respiratory tract through oxidation may take a few days. The biomarkers of systemic inflammation and hemostasis (i.e., fibrinogen, sCD62P, sCD40L, and vWF) showed the largest effect estimates at lag days 2 to 4 for $PM_{2.5}$ and most PM species. Based on this overall lag-day pattern for local (respiratory tract) and systemic events, it is reasonable to hypothesize that inhaled $PM_{2.5}$ deposits in the lung and rapidly triggers local inflammation and oxidative stress; these respiratory effects then induce systemic effects within a few days. However, it is important to note that a previous study found an effect of PM on platelet activation within 24 hours of exposure (Lucking et al. 2008).

The timing of systemic effects for TPN appears to be different from that for $PM_{2.5}$, with most of the systemic inflammatory and hemostasis biomarkers showing the largest effects at lag days 0 and 1. This can be explained by the difference in particle size between $PM_{2.5}$ and TPN. As shown in Figure 8, the period-average mass median particle diameters were between 363 nm to 423 nm, indicating that more than 50% of $PM_{2.5}$ mass concentration resulted from particles larger than 363 nm. In contrast, TPN concentrations were dominated by ultrafine particles (i.e., particles smaller than 100 nm), which may be able to enter the circulation system directly. This makes it biologically plausible that ultrafine particles directly exert systemic effects without prior mediation through pulmonary events. For the three gaseous pollutants (SO_2, CO, and NO_2), we did not observe a clear distinction in timing (lag days) between the respiratory-effect biomarkers and the systemic-effect biomarkers (see Table 8), suggesting that gaseous pollutants, unlike $PM_{2.5}$ mass, may not necessarily need to initiate pulmonary biochemical effects first before inducing systemic effects.

Some of the lag-day patterns are intriguing. For most biomarkers, there was a gradual increase in effect estimates in the early lag periods (lags 0–2), followed by a gradual decline in effect estimates to often negative effects (i.e., an increased pollutant concentration associated with decreased biomarker levels) in later lag periods. These negative effects could be due to compensatory mechanisms responding to the increases in the biomarker triggered by pollution at early lags. In the case of SBP (Figure 15), fibrinogen (Figure 16), and EBC nitrate (Figure 23), the direction of its association with pollutants appeared to bounce back and forth across lag days, suggesting this was simply reflecting random noise or perhaps a more complicated dynamic response. To have a better understanding of the timing, more sophisticated methods (e.g., physiologically based biokinetic modeling) are needed. Our initial observations, reported here, are intended to provide some insights that may lead to further understanding of the timing of biochemical events that underlie the adverse cardiorespiratory effects of air pollution through the mechanisms being examined in our study.

STRENGTHS, LIMITATIONS, AND SENSITIVITY ANALYSES

This study has several strengths including the combined use of a unique quasi-experimental "A-B-A" design and a panel-study approach and a large range of pollutant concentration measurements enabling enhanced power for detecting significant pollutant effects on biomarker levels. The vast majority of our subjects lived in dormitories where there were no cooking facilities, thus eliminating a major source of indoor air pollution. However, although our design ideally required that all study subjects reside and work on the Peking University First Hospital campus, in reality, although all the subjects indeed worked at the Hospital, only 8% (10 out of 125) lived in dormitories located on the hospital grounds. Most (105 out of 125, or 84%) of the subjects lived in the dormitories of Peking University Health Sciences Center, about 5 km away from the hospital; and the remaining 10 subjects (8%) lived in off-campus apartments that were not immediately adjacent to the hospital. Therefore, traffic exposure to air pollution during the commute for those who lived outside hospital grounds may not be captured accurately by pollutant concentrations measured at the fixed site in the center of the hospital campus. However, a sensitivity analysis including only the 105 subjects living on hospital grounds showed few changes in the results.

We did not measure pollutant concentrations at locations other than the hospital grounds, thereby possibly missing important exposures. However, this omission should result only in nondifferential exposure error and underestimates of pollutant-mediated biomarker changes, because the study design was based on within-person comparisons. In addition, we collected detailed time–activity data for each subject (see Appendix D), which will allow an assessment in the future of the potential impact of exposures associated with differential time–activities.

Our period-comparison ("A-B-A") approach may raise the question of whether unmeasured factors other than air pollutants contributed to the observed changes in biomarkers. For this reason, we selected medical students undergoing clinical training who had no lifestyle changes during the Olympics. We selected a relatively narrow time window to minimize potential seasonal confounding on the effects of air pollution. Our pre-Olympics and during-Olympics measurements all occurred in the summer months, but the post-Olympics measurements fell in the early autumn season. For this reason, we included moving averages (up to 7 days) of temperature and RH as indicators of season. We also conducted a set of sensitivity analyses to examine the potential impact of temperature, RH, and rainfall on the period-effect results as well as on the pollutant–biomarker associations, as discussed below.

We defined pre-, during-, and post-Olympics periods based on the timeline of the pollution control measures for the Olympics. Due to temporal variations in meteorology, there existed days within the during-Olympics period on which pollutant concentrations were higher than in the pre- and post-Olympics periods. This may have increased the width of the confidence intervals for our period estimates. Excluding these during-Olympics "high-pollution" days from the analysis, however, had little impact on our overall findings.

Regarding our sensitivity analyses related to meteorology, Appendix E summarizes the degrees of freedom selected for each meteorologic parameter, as well as the *P* values showing whether temperature and RH and their moving averages were significantly associated with each biomarker. For biomarkers for which these meteorologic parameters were not statistically significant, we used unadjusted single-pollutant models (without controlling for temperature and RH). For the biomarkers for which not all adjustments for temperature and RH were nonsignificant, we re-ran the models, controlling for only the significant meteorologic parameters (including moving averages). The results are summarized in Appendix J for the period analysis and Appendix K for the pollutant–biomarker analysis, with the significance given in Appendix E. When temperature and RH results that had no significant effect on biomarkers were deleted from the models, the overall pollutant–biomarker pattern of results remained very similar for most of these biomarkers with a few notable exceptions (e.g., DBP and SBP). For the period analysis, a comparison of Figure 10 and Appendix J shows a change only in statistical significance but not in direction for LF (HRV) from the pre-Olympics to the during-Olympics period and increases in estimated changes for some HRV indices (HF, LF, SDNN, and total power) from the during- to the post-Olympics period. As expected, when temperature or RH itself had a statistically significant effect on a biomarker, the effect estimates from temperature- and RH-adjusted analyses were typically smaller and/or less likely to be statistically significant compared with the effect estimates from nonadjusted analyses. For this reason, we report all of our main results based on the adjusted analyses to avoid attributing temperature or RH effects to pollutant effects.

Results from sensitivity analyses excluding rainy days are summarized in Appendix L. As was expected, given that only 8% of the days had >1 mm precipitation, the results did not change substantially. And, as expected, the standard errors of the estimates were in general larger due to the reduction in sample size.

In our primary analyses, we accounted for unexplained sources of residual confounding by seasonal differences

through the inclusion of moving averages of temperature and RH. It was expected that these moving averages would serve as proxies for things such as changes in social behavior due to changes in season as well as longer-term physiologic changes due to season. In addition, sensitivity analyses were conducted by excluding the post-Olympics period, because it represented the largest difference in seasonality. Both the pre- and during-Olympics periods occurred during the summer, and hence, we did not expect many seasonal differences between these two periods. Results from the single-pollutant models for the selected biomarkers when excluding the post-Olympics observations are summarized in Appendix M. Compared with the results from the analyses including all three periods, the biomarker that changed most notably was perhaps EBC nitrite, but this change was still not large enough to alter the overall finding on this biomarker. As expected, the reduced sample size in the two-period-only analysis resulted in wider confidence intervals for the effect estimates; however, it did not substantially change the main findings, confirming that it was unlikely that those findings were due to unmeasured factors associated with seasonal changes.

In an attempt to assess within-period effects of individual pollutants, we included the "period" indicator as a covariate in the single-pollutant models. Results from this set of analyses are shown in Appendix N for selected biomarkers. We observed, in general, reduced "within-period" effect estimates compared with the effect estimates when the "period" was not adjusted for, except for EBC nitrite+ nitrate for which this difference was less notable. The substantial attenuation, with little change in the confidence intervals, of the analyses with "period" indicator (Appendix N) was expected, given that changes in pollution levels were associated with period changes in this real-world quasi-experimental design. In the present study, we were mainly interested in finding pollutant–biomarker associations across a wide range of pollutant concentrations. In this regard, it is not necessary to adjust for the time period in the analyses. However, it may be interesting to explore the relative contributions of within-period versus between-period estimates to the overall effect estimates in future analyses.

Finally, the non-meteorologic sensitivity analysis using subject identification as a fixed effect showed attenuated effects of pollutants on biomarkers, but the trend remained largely the same as that seen with the effects estimated from the primary models using subject identification as a random effect (see Appendix O). This confirms the robustness of our primary models.

In summary, findings from these sensitivity analyses confirm that the statistical models used in our main analyses were robust. Changes in biomarker levels observed by period and in association with changes in pollutant concentrations were most likely driven by the air quality changes rather than seasonal differences in other unmeasured factors.

In this study, we measured a comprehensive battery of biomarkers reflecting multiple pathways. Many of these biomarkers (e.g., urinary 8-OHdG and EBC nitrite and nitrate) were measured using state-of-the-art analytical chemistry techniques. However, because of field resource constraints, some biomarkers (e.g., CRP, platelet aggregation, and EBC 8-isoprostane) were measured using "clinical-grade" methods, which were less sensitive or less accurate than "research-grade" analytical methods. Measurements of HRV over 10-minute periods are known to be far less sensitive than measurements taken over 24 hours (using a Holter ECG monitor), which would include measurements during all kinds of activities. However, requiring subjects to wear a monitor would have resulted in substantial recruiting difficulties.

In this study, we also measured a large suite of air pollutants including $PM_{2.5}$, many $PM_{2.5}$ constituents, size-resolved particle number concentrations, and commonly measured gases (SO_2, CO, and NO_2). However, particle number concentrations were measured from a separate site about 7 km away. Spatial variability in particle number may depend on specific particle size; and particle size may determine how far and how efficiently particles travel within and beyond the respiratory tract. Hence, future analyses should consider use of size-specific concentrations rather than the TPN measurements used in the current analysis.

Another obvious limitation of this report is the lack of more in-depth analysis of some important $PM_{2.5}$ constituents, such as transition metals and PAHs, in relation to biomarkers, due to time and resource constraints. It would be interesting to investigate in future analysis whether changes in $PM_{2.5}$ composition modify $PM_{2.5}$ effects.

We examined the effects of various pollutants over a range of time windows (lag days 0 to 6). These analyses have generated some initial insights about whether and how different pollutants may affect different biomarkers within different time periods, which may lead to further understanding of the mechanisms involved. Future analyses of hourly data and PM speciation data may produce additional insights.

Unlike many previous studies of more susceptible subjects, such as children and older adults with or without preexisting cardiorespiratory disease, the present study used a homogeneous group of young adults who were free of any cardiorespiratory or other chronic diseases, had

similar lifestyles, and were all ethnic Han Chinese. We observed statistically significant biomarker changes, in these young and healthy individuals, in response both to the air quality intervention (in our period comparisons) and to day-to-day changes in pollutant concentrations (in our pollutant–biomarker association analyses). Aside from blood pressure, these biomarker changes have not previously been shown to be cardiorespiratory risk factors and are not presently recognized as mediating or initiating clinical events. As such, our findings greatly increase understanding of the mechanisms by which air pollution enhances cardiorespiratory risk.

Compared with the "average" population, the medical residents measured in this study may have spent less time outdoors and more time in an indoor air-conditioned environment during work hours. This would have tended to lessen exposure to ambient air pollution and consequently lessen the impact of the changes in pollution concentrations during the Olympics.

The four mechanisms (autonomic dysfunction, oxidative stress, inflammation, and hemostasis) that were investigated in our study may be interrelated. For example, ROS characterizing oxidative stress may induce inflammation, and inflammatory mediators may engage in biochemical reactions that produce ROS. Inflammation contributes to the instability of pre-existing atherosclerotic plaques, and thrombosis, which is dependent on platelet activation, is responsible for arterial obstruction on top of any such ruptured plaques (hemostasis). Multiple biomarkers, such as were measured in this study, for each of these mechanisms may facilitate future investigations of any relationships among the mechanistic pathways related to the air pollution effects. As a starting point, we performed a simple analysis to examine correlations among the biomarkers measured in this study (see Appendix H). Since it is clear that HRV indices were moderately to highly correlated with each other, interpretation of the relations between the other biomarkers, even within the same mechanistic pathway, is not straightforward. There exist statistical and methodologic challenges at the present time to better addressing this issue. Statistical models incorporating biokinetics and/or biodynamics may help us better understand the complicated physiologic and biochemical processes involving the biomarkers measured in the current study.

CONCLUSIONS

Taking advantage of a unique opportunity during which the Chinese government mandated temporary closures or relocations of industry and reductions in motor vehicle use during the 2008 Beijing Olympics, we examined whether there were reductions in pollutant concentrations and improvements in biomarkers reflecting pathophysiologic pathways hypothesized to underlie epidemiologic associations between ambient air pollution and cardiorespiratory morbidity and mortality. We observed large (up to 60%) reductions in pollutants (except O_3) during the Olympics pollution intervention period compared with the pre-Olympics period. Concomitantly, in a panel of healthy, young, and nonsmoking adults, we observed salutary changes, many of them statistically significant, in measures of cardiovascular physiology (HR and SBP), biomarkers of pulmonary inflammation and oxidative stress (FeNO; EBC nitrite, nitrate, nitrite+nitrate, and pH; and 8-isoprostane), biomarkers of systemic inflammation and oxidative stress (fibrinogen, vWF, and urinary 8-OHdG), and biomarkers of hemostasis (including biomarkers of platelet activation [sCD40L and sCD62P] and vWF). In the post-Olympics period, when the pollution control measures were relaxed, mean concentrations for most of the pollutants (except O_3 and SO_4^{2-} in $PM_{2.5}$) increased, and the improvement of most biomarkers reversed. Consistent with these findings from the period comparisons, the biomarkers that showed significant period changes also showed significant associations with multiple pollutants after we adjusted for meteorologic parameters and after we considered other potential confounders in the sensitivity analyses. For most of the biomarkers the associations were in the direction (an increase or decrease with increases in pollutant concentration) hypothesized, providing further evidence that the period effects were due to changes in air quality, independent of season and meteorologic conditions.

These findings suggest that air pollution acutely and adversely affects cardiorespiratory health via pulmonary and systemic inflammation, oxidative stress, and hemostasis, and by increasing HR and blood pressure, in healthy young adults. These findings are of uncertain clinical significance at the present time.

IMPLICATION OF FINDINGS

Epidemiologic studies showing associations between air pollution and cardiorespiratory mortality and morbidity have been largely conducted in the elderly and those with cardiorespiratory diseases, but we observed biomarker changes linking air pollution with disease pathways in a homogeneous group of healthy young adults who had similar lifestyles and no known indoor exposures. This, along with the fact that our findings are supported by both a quasi-experimental analysis (period comparison) and a

panel-study analysis (pollutant–biomarker associations), greatly increases the certainty in the mechanisms or pathways by which air pollution affects cardiorespiratory health, independent of commonly recognized risk factors (e.g., age and predisposing diseases).

Our findings have broad public health implications, suggesting that improvements in air quality not only benefit susceptible populations as shown in previous studies, but also can reduce the body burden of oxidative stress, inflammation, and blood coagulation, as well as lower HR and SBP, in healthy young people. Because the pathways under study are thought to play a role in many other diseases, even natural aging, our findings provide mechanistic data to possibly support a previous assessment that air quality improvement over the last decades, thanks to air pollution regulations, accounts for as much as a 15% overall increase in life expectancy (some 5 to 10 months) in U.S. metropolitan areas (Pope et al. 2009). Given that current air pollution levels in many megacities, such as Beijing, are similar to those measured in U.S. cities before air quality regulations were enacted (HEI 2010), aggressive interventions are likely to have public health benefits. Salutary changes in biomarkers, including two well-established cardiovascular risk factors (HR and BP) observed in this study, support confidence in the immediate efficacy of actions to improve air quality. On the other hand, the reversed biomarker changes after the Olympics suggest that sustained air pollution interventions will be necessary to continue public health benefits.

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APPENDIX A. $PM_{2.5}$ Constituent Concentration Summary Statistics

Table A.1. Ions in $PM_{2.5}$[a]

Pollutant / Period	*n*	Mean	SD	Min	Median	Max	IQR
Na^+ (μg/m^3)							
Whole period	92	2.5×10^{-1}	1.6×10^{-1}	2.0×10^{-2}	2.3×10^{-1}	7.7×10^{-1}	1.7×10^{-1}
Pre-Olympics	35	2.8×10^{-1}	1.2×10^{-1}	8.0×10^{-2}	2.7×10^{-1}	6.5×10^{-1}	1.3×10^{-1}
During-Olympics	28	1.8×10^{-1}	1.2×10^{-1}	2.0×10^{-2}	1.4×10^{-1}	5.0×10^{-1}	1.8×10^{-1}
Post-Olympics	29	2.8×10^{-1}	2.1×10^{-1}	4.0×10^{-2}	2.2×10^{-1}	7.7×10^{-1}	3.4×10^{-1}
NH_4^+ (μg/m^3)							
Whole period	92	1.4×10^{1}	1.1×10^{1}	2.0×10^{-1}	1.3×10^{1}	4.8×10^{1}	1.9×10^{1}
Pre-Olympics	35	1.9×10^{1}	9.8×10^{0}	2.3×10^{0}	1.8×10^{1}	4.8×10^{1}	1.1×10^{1}
During-Olympics	28	1.3×10^{1}	1.1×10^{1}	7.8×10^{-1}	1.1×10^{1}	4.2×10^{1}	1.8×10^{1}
Post-Olympics	29	8.3×10^{0}	1.0×10^{1}	2.0×10^{-1}	2.5×10^{0}	3.4×10^{1}	1.2×10^{1}
K^+ (μg/m^3)							
Whole period	92	1.2×10^{0}	9.5×10^{-1}	9.0×10^{-2}	9.0×10^{-1}	4.8×10^{0}	1.4×10^{0}
Pre-Olympics	35	1.7×10^{0}	1.1×10^{0}	3.7×10^{-1}	1.6×10^{0}	4.8×10^{0}	1.3×10^{0}
During-Olympics	28	7.0×10^{-1}	4.5×10^{-1}	9.0×10^{-2}	6.6×10^{-1}	2.0×10^{0}	6.5×10^{-1}
Post-Olympics	29	1.1×10^{0}	8.5×10^{-1}	1.4×10^{-1}	8.2×10^{-1}	2.8×10^{0}	1.5×10^{0}
Mg^{2+} (μg/m^3)							
Whole period	92	5.0×10^{-2}	6.0×10^{-2}	1.0×10^{-2}	4.0×10^{-2}	3.8×10^{-1}	3.0×10^{-2}
Pre-Olympics	35	4.0×10^{-2}	2.0×10^{-2}	1.0×10^{-2}	4.0×10^{-2}	1.1×10^{-1}	2.0×10^{-2}
During-Olympics	28	4.0×10^{-2}	2.0×10^{-2}	1.0×10^{-2}	3.0×10^{-2}	9.0×10^{-2}	2.0×10^{-2}
Post-Olympics	29	9.0×10^{-2}	9.0×10^{-2}	2.0×10^{-2}	6.0×10^{-2}	3.8×10^{-1}	4.0×10^{-2}
Ca^{2+} (μg/m^3)							
Whole period	92	6.0×10^{-1}	8.9×10^{-1}	1.0×10^{-1}	4.2×10^{-1}	6.0×10^{0}	3.0×10^{-1}
Pre-Olympics	35	4.3×10^{-1}	2.8×10^{-1}	1.2×10^{-1}	3.8×10^{-1}	1.7×10^{0}	2.7×10^{-1}
During-Olympics	28	3.5×10^{-1}	1.5×10^{-1}	1.0×10^{-1}	3.3×10^{-1}	6.7×10^{-1}	2.0×10^{-1}
Post-Olympics	29	1.1×10^{0}	1.5×10^{0}	1.2×10^{-1}	6.3×10^{-1}	6.0×10^{0}	3.6×10^{-1}
F^- (μg/m^3)							
Whole period	92	2.0×10^{-2}	3.0×10^{-2}	0.0×10^{0}	1.0×10^{-2}	2.2×10^{-1}	2.0×10^{-2}
Pre-Olympics	35	2.0×10^{-2}	1.0×10^{-2}	0.0×10^{0}	1.0×10^{-2}	8.0×10^{-2}	1.0×10^{-2}
During-Olympics	28	1.0×10^{-2}	1.0×10^{-2}	0.0×10^{0}	1.0×10^{-2}	3.0×10^{-2}	1.0×10^{-2}
Post-Olympics	29	4.0×10^{-2}	5.0×10^{-2}	0.0×10^{0}	3.0×10^{-2}	2.2×10^{-1}	2.0×10^{-2}
Cl^- (μg/m^3)							
Whole period	92	9.3×10^{-1}	8.8×10^{-1}	2.0×10^{-2}	7.0×10^{-1}	3.5×10^{0}	1.1×10^{0}
Pre-Olympics	35	1.3×10^{0}	9.9×10^{-1}	8.0×10^{-2}	1.2×10^{0}	3.5×10^{0}	9.8×10^{-1}
During-Olympics	28	4.5×10^{-1}	5.1×10^{-1}	2.0×10^{-2}	2.3×10^{-1}	2.3×10^{0}	6.1×10^{-1}
Post-Olympics	29	8.9×10^{-1}	8.1×10^{-1}	5.0×10^{-2}	6.9×10^{-1}	2.5×10^{0}	1.1×10^{0}
NO_3^- (μg/m^3)							
Whole period	92	1.5×10^{1}	1.3×10^{1}	1.8×10^{-1}	1.2×10^{1}	5.5×10^{1}	1.8×10^{1}
Pre-Olympics	35	1.9×10^{1}	1.0×10^{1}	1.7×10^{0}	1.8×10^{1}	4.0×10^{1}	1.6×10^{1}
During-Olympics	28	9.1×10^{0}	7.2×10^{0}	1.8×10^{-1}	7.4×10^{0}	2.3×10^{1}	1.1×10^{1}
Post-Olympics	29	1.7×10^{1}	1.9×10^{1}	3.7×10^{-1}	6.1×10^{0}	5.5×10^{1}	2.7×10^{1}
SO_4^{2-} (μg/m^3)							
Whole period	92	2.2×10^{1}	1.7×10^{1}	9.5×10^{-1}	2.1×10^{1}	7.4×10^{1}	2.8×10^{1}
Pre-Olympics	35	2.8×10^{1}	1.3×10^{1}	5.4×10^{0}	3.0×10^{1}	6.5×10^{1}	1.7×10^{1}
During-Olympics	28	2.3×10^{1}	1.9×10^{1}	2.0×10^{0}	2.1×10^{1}	7.4×10^{1}	3.2×10^{1}
Post-Olympics	29	1.2×10^{1}	1.5×10^{1}	9.5×10^{-1}	4.8×10^{0}	4.8×10^{1}	1.6×10^{1}

[a] All samples were above detection limit. Summary statistics are based on 24-hour averages (from ~10 AM to ~10 AM next day).

Table A.2. PAHs in $PM_{2.5}$[a]

Pollutant / Period	*n*	%[b]	Mean	SD	Min	Median	Max	IQR
Acenaphthylene (ng/m^3)								
Whole period	94	94	3.4	3.0	0.0	2.3	15.3	3.3
Pre-Olympics	35	83	4.9	3.7	0.0	4.8	15.3	4.7
During-Olympics	28	100	3.6	2.6	1.0	2.7	11.7	2.6
Post-Olympics	31	100	1.6	0.9	0.5	1.5	5.7	1.0
Fluorene (ng/m^3)								
Whole period	94	89	0.6	0.6	0.0	0.7	2.3	1.0
Pre-Olympics	35	74	1.0	0.7	0.0	1.3	2.3	1.3
During-Olympics	28	96	0.8	0.3	0.0	0.8	1.2	0.4
Post-Olympics	31	100	0.1	0.0	0.1	0.1	0.3	0.0
Phenanthrene (ng/m^3)								
Whole period	94	100	1.6	1.1	0.2	1.5	4.8	1.9
Pre-Olympics	35	100	2.5	0.8	1.0	2.4	4.8	0.8
During-Olympics	28	100	1.7	0.5	1.0	1.6	2.5	0.8
Post-Olympics	31	100	0.4	0.1	0.2	0.3	0.6	0.2
Fluoranthene (ng/m^3)								
Whole period	94	100	4.8	3.7	0.5	3.4	17.6	4.7
Pre-Olympics	35	100	7.8	3.7	2.1	7.0	17.6	3.5
During-Olympics	28	100	4.3	2.4	1.2	3.6	11.1	3.1
Post-Olympics	31	100	1.8	0.9	0.5	1.8	3.5	1.5
Pyrene (ng/m^3)								
Whole period	94	98	2.0	1.5	0.0	1.7	6.7	1.9
Pre-Olympics	35	100	3.2	1.5	1.0	2.9	6.7	1.6
During-Olympics	28	93	1.8	1.1	0.0	1.7	4.8	1.4
Post-Olympics	31	100	0.8	0.4	0.2	0.8	1.5	0.6
Benzo[*a*]anthracene (ng/m^3)								
Whole period	94	97	1.4	1.0	0.0	1.2	4.8	1.1
Pre-Olympics	35	91	1.9	1.2	0.0	1.7	4.8	1.3
During-Olympics	28	100	1.1	0.8	0.2	0.9	3.3	1.0
Post-Olympics	31	100	1.1	0.6	0.2	1.0	2.6	0.9
Chrysene (ng/m^3)								
Whole period	94	100	2.6	2.0	0.2	2.0	8.8	2.4
Pre-Olympics	35	100	4.2	2.0	1.1	3.8	8.8	2.1
During-Olympics	28	100	2.4	1.8	0.3	2.0	6.9	1.7
Post-Olympics	31	100	1.2	0.6	0.2	1.2	2.7	0.9

(*Table continues on next page*)

[a] Summary statistics are based on 24-hour averages (from ~10 AM to ~10 AM next day).
[b] Percent of observations above detection limit.

Table A.2 (*Continued*). PAHs in $PM_{2.5}$[a]

Pollutant / Period	*n*	%[b]	Mean	SD	Min	Median	Max	IQR
Benzo[*b*]fluoranthene (ng/m^3)								
Whole period	94	100	13.2	9.9	0.7	10.0	45.6	10.5
Pre-Olympics	35	100	20.0	10.2	4.2	17.4	45.6	10.1
During-Olympics	28	100	12.1	8.9	2.8	10.4	37.3	10.6
Post-Olympics	31	100	6.5	3.5	0.7	7.4	17.2	4.8
Benzo[*k*]fluoranthene (ng/m^3)								
Whole period	94	99	1.5	0.9	0.0	1.4	4.5	1.1
Pre-Olympics	35	97	1.9	1.0	0.0	1.8	4.5	1.1
During-Olympics	28	100	1.1	0.8	0.3	1.0	3.4	0.8
Post-Olympics	31	100	1.4	0.7	0.2	1.5	3.5	1.0
Benzo[*e*]pyrene (ng/m^3)								
Whole period	94	100	5.5	4.9	0.1	4.3	20.9	6.3
Pre-Olympics	35	100	9.0	4.6	1.7	7.8	20.9	4.6
During-Olympics	28	100	5.8	4.4	1.4	4.9	19.4	5.0
Post-Olympics	31	100	1.2	0.7	0.1	1.2	3.0	0.9
Benzo[*a*]pyrene (ng/m^3)								
Whole period	94	100	2.7	1.6	0.3	2.6	7.5	2.2
Pre-Olympics	35	100	3.0	1.6	0.6	2.6	6.7	1.7
During-Olympics	28	100	1.9	1.4	0.3	1.6	6.4	1.9
Post-Olympics	31	100	3.1	1.5	0.4	3.5	7.5	2.0
Indeno[1,2,3-*cd*]pyrene (ng/m^3)								
Whole period	94	100	4.2	2.8	0.2	3.5	13.8	3.6
Pre-Olympics	35	100	5.6	3.0	1.1	5.7	13.8	3.2
During-Olympics	28	100	3.7	2.7	0.9	3.2	12.9	3.5
Post-Olympics	31	100	2.9	1.8	0.2	2.8	8.7	2.2
Dibenzo[*a*,*h*]anthracene (ng/m^3)								
Whole period	94	91	0.6	1.1	0.0	0.3	8.8	0.5
Pre-Olympics	35	77	1.2	1.7	0.0	1.2	8.8	1.6
During-Olympics	28	100	0.2	0.2	0.0	0.1	0.9	0.2
Post-Olympics	31	100	0.4	0.3	0.0	0.4	1.3	0.3
Benzo[*g*,*h*,*i*]perylene (μg/m^3)								
Whole period	94	99	6.2	4.4	0.0	5.2	21.6	5.8
Pre-Olympics	35	97	8.7	4.8	0.0	9.1	21.6	5.2
During-Olympics	28	100	5.9	4.2	1.5	5.0	20.0	5.2
Post-Olympics	31	100	3.8	2.1	0.2	3.6	10.1	2.4

[a] Summary statistics are based on 24-hour averages (from ~10 AM to ~10 AM next day).
[b] Percent of observations above detection limit.

Table A.3. Elements in $PM_{2.5}$[a]

Pollutant / Period	*n*	%	Mean	SD	Min	Median	Max	IQR
Na (μg/m^3)								
Whole period	94	100	3.8×10^{-1}	2.1×10^{-1}	9.0×10^{-2}	3.4×10^{-1}	1.1×10^{0}	2.5×10^{-1}
Pre-Olympics	35	100	3.8×10^{-1}	1.3×10^{-1}	1.1×10^{-1}	3.6×10^{-1}	6.7×10^{-1}	1.4×10^{-1}
During-Olympics	28	100	3.1×10^{-1}	1.7×10^{-1}	9.0×10^{-2}	2.7×10^{-1}	7.3×10^{-1}	2.2×10^{-1}
Post-Olympics	31	100	4.5×10^{-1}	2.9×10^{-1}	9.1×10^{-2}	3.1×10^{-1}	1.1×10^{0}	4.5×10^{-1}
Mg (μg/m^3)								
Whole period	94	100	2.2×10^{-1}	1.7×10^{-1}	3.1×10^{-2}	2.0×10^{-1}	1.2×10^{0}	1.4×10^{-1}
Pre-Olympics	35	100	2.2×10^{-1}	1.4×10^{-1}	3.1×10^{-2}	2.1×10^{-1}	7.4×10^{-1}	1.1×10^{-1}
During-Olympics	28	100	1.7×10^{-1}	7.7×10^{-2}	4.1×10^{-2}	1.6×10^{-1}	3.1×10^{-1}	1.1×10^{-1}
Post-Olympics	31	100	2.6×10^{-1}	2.4×10^{-1}	6.4×10^{-2}	2.0×10^{-1}	1.2×10^{0}	1.5×10^{-1}
Al (μg/m^3)								
Whole period	94	100	3.2×10^{-1}	4.0×10^{-1}	1.8×10^{-2}	2.3×10^{-1}	2.8×10^{0}	2.6×10^{-1}
Pre-Olympics	35	100	3.1×10^{-1}	2.5×10^{-1}	6.5×10^{-2}	2.5×10^{-1}	1.6×10^{0}	1.2×10^{-1}
During-Olympics	28	100	8.1×10^{-2}	7.3×10^{-2}	1.8×10^{-2}	5.6×10^{-2}	4.0×10^{-1}	6.7×10^{-2}
Post-Olympics	31	100	5.6×10^{-1}	5.6×10^{-1}	1.1×10^{-1}	4.0×10^{-1}	2.8×10^{0}	3.1×10^{-1}
P (μg/m^3)								
Whole period	94	90	4.6×10^{-2}	3.6×10^{-2}	7.9×10^{-5}	4.6×10^{-2}	1.5×10^{-1}	4.7×10^{-2}
Pre-Olympics	35	74	2.3×10^{-2}	2.6×10^{-2}	7.9×10^{-5}	2.1×10^{-2}	9.2×10^{-2}	3.6×10^{-2}
During-Olympics	28	100	6.4×10^{-2}	3.5×10^{-2}	1.7×10^{-2}	5.1×10^{-2}	1.4×10^{-1}	4.3×10^{-2}
Post-Olympics	31	100	5.6×10^{-2}	3.2×10^{-2}	1.0×10^{-2}	4.8×10^{-2}	1.5×10^{-1}	4.7×10^{-2}
K (μg/m^3)								
Whole period	94	100	1.3×10^{0}	9.5×10^{-1}	1.3×10^{-1}	1.0×10^{0}	5.0×10^{0}	1.2×10^{0}
Pre-Olympics	35	100	1.7×10^{0}	1.1×10^{0}	3.4×10^{-1}	1.7×10^{0}	5.0×10^{0}	1.4×10^{0}
During-Olympics	28	100	7.6×10^{-1}	4.1×10^{-1}	1.3×10^{-1}	7.4×10^{-1}	1.6×10^{0}	5.6×10^{-1}
Post-Olympics	31	100	1.4×10^{0}	9.2×10^{-1}	2.6×10^{-1}	1.2×10^{0}	3.7×10^{0}	1.4×10^{0}
Ca (μg/m^3)								
Whole period	94	100	6.7×10^{-1}	6.1×10^{-1}	1.0×10^{-1}	5.7×10^{-1}	4.9×10^{0}	3.6×10^{-1}
Pre-Olympics	35	100	5.8×10^{-1}	3.0×10^{-1}	1.0×10^{-1}	5.6×10^{-1}	1.8×10^{0}	2.1×10^{-1}
During-Olympics	28	100	4.7×10^{-1}	2.2×10^{-1}	1.0×10^{-1}	4.5×10^{-1}	9.4×10^{-1}	2.8×10^{-1}
Post-Olympics	31	100	9.5×10^{-1}	9.4×10^{-1}	2.6×10^{-1}	6.6×10^{-1}	4.9×10^{0}	4.9×10^{-1}
Ti (μg/m^3)								
Whole period	94	100	3.8×10^{-2}	3.1×10^{-2}	7.3×10^{-3}	3.0×10^{-2}	2.3×10^{-1}	1.8×10^{-2}
Pre-Olympics	35	100	3.1×10^{-2}	2.1×10^{-2}	7.3×10^{-3}	2.7×10^{-2}	1.2×10^{-1}	1.3×10^{-2}
During-Olympics	28	100	3.1×10^{-2}	1.4×10^{-2}	1.1×10^{-2}	2.8×10^{-2}	6.2×10^{-2}	1.8×10^{-2}
Post-Olympics	31	100	5.2×10^{-2}	4.6×10^{-2}	1.6×10^{-2}	3.8×10^{-2}	2.3×10^{-1}	2.8×10^{-2}
V (μg/m^3)								
Whole period	94	99	2.9×10^{-3}	3.0×10^{-3}	5.2×10^{-7}	2.0×10^{-3}	2.4×10^{-2}	2.6×10^{-3}
Pre-Olympics	35	100	4.6×10^{-3}	4.1×10^{-3}	8.9×10^{-4}	3.6×10^{-3}	2.4×10^{-2}	3.3×10^{-3}
During-Olympics	28	100	2.0×10^{-3}	1.2×10^{-3}	2.3×10^{-4}	1.6×10^{-3}	4.9×10^{-3}	1.9×10^{-3}
Post-Olympics	31	97	1.9×10^{-3}	1.6×10^{-3}	5.2×10^{-7}	1.3×10^{-3}	6.7×10^{-3}	1.4×10^{-3}

(*Table continues on next page*)

[a] Summary statistics are based on 24-hour averages (from ~10 AM to ~10 AM next day).

Table A.3 (*Continued*). Elements in $PM_{2.5}$[a]

Pollutant / Period	*n*	%	Mean	SD	Min	Median	Max	IQR
Cr ($\mu g/m^3$)								
Whole period	94	100	5.1×10^{-3}	3.7×10^{-3}	6.6×10^{-4}	4.0×10^{-3}	2.2×10^{-2}	3.3×10^{-3}
Pre-Olympics	35	100	3.1×10^{-3}	1.3×10^{-3}	6.6×10^{-4}	2.9×10^{-3}	5.6×10^{-3}	1.6×10^{-3}
During-Olympics	28	100	5.0×10^{-3}	2.4×10^{-3}	1.5×10^{-3}	4.5×10^{-3}	1.2×10^{-2}	3.4×10^{-3}
Post-Olympics	31	100	7.4×10^{-3}	5.1×10^{-3}	2.0×10^{-3}	5.9×10^{-3}	2.2×10^{-2}	7.7×10^{-3}
Mn ($\mu g/m^3$)								
Whole period	94	100	4.3×10^{-2}	2.6×10^{-2}	9.4×10^{-3}	3.5×10^{-2}	1.6×10^{-1}	2.4×10^{-2}
Pre-Olympics	35	100	4.6×10^{-2}	2.5×10^{-2}	1.4×10^{-2}	4.1×10^{-2}	1.6×10^{-1}	2.0×10^{-2}
During-Olympics	28	100	2.8×10^{-2}	1.2×10^{-2}	9.4×10^{-3}	2.9×10^{-2}	5.6×10^{-2}	1.5×10^{-2}
Post-Olympics	31	100	5.3×10^{-2}	3.0×10^{-2}	1.2×10^{-2}	4.5×10^{-2}	1.2×10^{-1}	3.6×10^{-2}
Fe ($\mu g/m^3$)								
Whole period	94	99	6.1×10^{-1}	4.5×10^{-1}	1.7×10^{-6}	5.1×10^{-1}	2.8×10^{0}	3.8×10^{-1}
Pre-Olympics	35	100	6.3×10^{-1}	3.5×10^{-1}	1.5×10^{-1}	5.3×10^{-1}	1.8×10^{0}	3.7×10^{-1}
During-Olympics	28	96	3.5×10^{-1}	2.8×10^{-1}	1.7×10^{-6}	3.1×10^{-1}	1.3×10^{0}	3.2×10^{-1}
Post-Olympics	31	100	8.1×10^{-1}	5.7×10^{-1}	3.0×10^{-1}	6.2×10^{-1}	2.8×10^{0}	4.9×10^{-1}
Co ($\mu g/m^3$)								
Whole period	94	94	1.4×10^{-3}	2.3×10^{-3}	1.7×10^{-7}	7.1×10^{-4}	1.4×10^{-2}	1.2×10^{-3}
Pre-Olympics	35	100	1.4×10^{-3}	2.0×10^{-3}	1.8×10^{-4}	7.2×10^{-4}	9.1×10^{-3}	8.4×10^{-4}
During-Olympics	28	79	4.3×10^{-4}	9.9×10^{-4}	1.7×10^{-7}	1.4×10^{-4}	4.2×10^{-3}	3.0×10^{-4}
Post-Olympics	31	100	2.4×10^{-3}	3.0×10^{-3}	2.5×10^{-4}	1.2×10^{-3}	1.4×10^{-2}	1.9×10^{-3}
Ni ($\mu g/m^3$)								
Whole period	94	83	2.2×10^{-3}	2.2×10^{-3}	4.7×10^{-7}	1.9×10^{-3}	1.3×10^{-2}	2.6×10^{-3}
Pre-Olympics	35	100	2.9×10^{-3}	2.4×10^{-3}	2.2×10^{-4}	2.2×10^{-3}	1.3×10^{-2}	2.1×10^{-3}
During-Olympics	28	43	3.5×10^{-4}	8.1×10^{-4}	4.7×10^{-7}	3.4×10^{-4}	3.2×10^{-3}	2.7×10^{-4}
Post-Olympics	31	100	3.0×10^{-3}	2.1×10^{-3}	1.0×10^{-3}	2.1×10^{-3}	8.4×10^{-3}	2.3×10^{-3}
Cu ($\mu g/m^3$)								
Whole period	94	99	3.4×10^{-2}	2.6×10^{-2}	3.9×10^{-7}	2.8×10^{-2}	1.4×10^{-1}	3.3×10^{-2}
Pre-Olympics	35	100	3.7×10^{-2}	1.4×10^{-2}	7.3×10^{-3}	3.5×10^{-2}	5.9×10^{-2}	2.2×10^{-2}
During-Olympics	28	96	2.7×10^{-2}	2.1×10^{-2}	3.9×10^{-7}	2.3×10^{-2}	7.5×10^{-2}	2.7×10^{-2}
Post-Olympics	31	100	3.7×10^{-2}	3.7×10^{-2}	5.3×10^{-3}	2.2×10^{-2}	1.4×10^{-1}	4.2×10^{-2}
Zn ($\mu g/m^3$)								
Whole period	94	100	2.3×10^{-1}	2.0×10^{-1}	5.6×10^{-3}	1.8×10^{-1}	1.1×10^{0}	2.8×10^{-1}
Pre-Olympics	35	100	3.4×10^{-1}	1.8×10^{-1}	5.8×10^{-2}	3.2×10^{-1}	1.1×10^{0}	2.0×10^{-1}
During-Olympics	28	100	4.1×10^{-2}	4.9×10^{-2}	5.6×10^{-3}	2.5×10^{-2}	2.6×10^{-1}	3.6×10^{-2}
Post-Olympics	31	100	2.7×10^{-1}	1.9×10^{-1}	4.7×10^{-2}	1.8×10^{-1}	8.4×10^{-1}	2.5×10^{-1}
As ($\mu g/m^3$)								
Whole period	94	100	9.7×10^{-3}	9.5×10^{-3}	3.3×10^{-4}	7.9×10^{-3}	4.7×10^{-2}	1.1×10^{-2}
Pre-Olympics	35	100	1.2×10^{-2}	5.8×10^{-3}	2.4×10^{-3}	1.2×10^{-2}	2.9×10^{-2}	5.7×10^{-3}
During-Olympics	28	100	1.9×10^{-3}	2.1×10^{-3}	3.3×10^{-4}	1.1×10^{-3}	1.0×10^{-2}	1.6×10^{-3}
Post-Olympics	31	100	1.4×10^{-2}	1.2×10^{-2}	8.5×10^{-4}	1.0×10^{-2}	4.7×10^{-2}	1.6×10^{-2}

(*Table continues on next page*)

[a] Summary statistics are based on 24-hour averages (from ~10 AM to ~10 AM next day).

Table A.3 (*Continued*). Elements in $PM_{2.5}$[a]

Pollutant / Period	*n*	%	Mean	SD	Min	Median	Max	IQR
Se (μg/m^3)								
Whole period	94	100	6.6×10^{-3}	4.7×10^{-3}	4.4×10^{-4}	6.5×10^{-3}	2.1×10^{-2}	6.6×10^{-3}
Pre-Olympics	35	100	8.8×10^{-3}	2.9×10^{-3}	3.5×10^{-3}	8.2×10^{-3}	1.5×10^{-2}	3.4×10^{-3}
During-Olympics	28	100	2.2×10^{-3}	1.9×10^{-3}	4.4×10^{-4}	1.7×10^{-3}	1.1×10^{-2}	1.3×10^{-3}
Post-Olympics	31	100	8.2×10^{-3}	5.4×10^{-3}	2.0×10^{-3}	6.8×10^{-3}	2.1×10^{-2}	6.4×10^{-3}
Mo (μg/m^3)								
Whole period	94	99	1.1×10^{-3}	1.1×10^{-3}	3.5×10^{-7}	8.9×10^{-4}	1.0×10^{-2}	7.9×10^{-4}
Pre-Olympics	35	100	9.4×10^{-4}	4.2×10^{-4}	2.7×10^{-4}	8.8×10^{-4}	2.2×10^{-3}	3.3×10^{-4}
During-Olympics	28	96	8.5×10^{-4}	6.5×10^{-4}	3.5×10^{-7}	7.8×10^{-4}	3.2×10^{-3}	7.3×10^{-4}
Post-Olympics	31	100	1.5×10^{-3}	1.7×10^{-3}	2.0×10^{-4}	1.1×10^{-3}	1.0×10^{-2}	1.4×10^{-3}
Cd (μg/m^3)								
Whole period	94	100	1.8×10^{-3}	1.5×10^{-3}	1.7×10^{-4}	1.5×10^{-3}	1.0×10^{-2}	1.5×10^{-3}
Pre-Olympics	35	100	1.7×10^{-3}	8.0×10^{-4}	2.5×10^{-4}	1.6×10^{-3}	4.1×10^{-3}	9.4×10^{-4}
During-Olympics	28	100	1.3×10^{-3}	8.6×10^{-4}	2.1×10^{-4}	1.0×10^{-3}	3.1×10^{-3}	1.4×10^{-3}
Post-Olympics	31	100	2.4×10^{-3}	2.2×10^{-3}	1.7×10^{-4}	1.8×10^{-3}	1.0×10^{-2}	2.0×10^{-3}
Ba (μg/m^3)								
Whole period	94	100	1.4×10^{-2}	1.3×10^{-2}	7.7×10^{-4}	9.1×10^{-3}	7.6×10^{-2}	8.5×10^{-3}
Pre-Olympics	35	100	9.9×10^{-3}	9.0×10^{-3}	1.9×10^{-3}	8.4×10^{-3}	5.5×10^{-2}	3.7×10^{-3}
During-Olympics	28	100	1.3×10^{-2}	1.4×10^{-2}	7.7×10^{-4}	8.9×10^{-3}	7.6×10^{-2}	1.1×10^{-2}
Post-Olympics	31	100	1.8×10^{-2}	1.5×10^{-2}	4.9×10^{-3}	1.4×10^{-2}	7.5×10^{-2}	1.4×10^{-2}
Tl (μg/m^3)								
Whole period	94	100	1.3×10^{-3}	9.1×10^{-4}	1.5×10^{-4}	1.1×10^{-3}	5.4×10^{-3}	9.2×10^{-4}
Pre-Olympics	35	100	1.3×10^{-3}	5.6×10^{-4}	1.8×10^{-4}	1.3×10^{-3}	2.5×10^{-3}	6.7×10^{-4}
During-Olympics	28	100	1.0×10^{-3}	6.7×10^{-4}	1.8×10^{-4}	7.8×10^{-4}	2.7×10^{-3}	7.7×10^{-4}
Post-Olympics	31	100	1.5×10^{-3}	1.3×10^{-3}	1.5×10^{-4}	1.1×10^{-3}	5.4×10^{-3}	1.2×10^{-3}
Pb (μg/m^3)								
Whole period	94	100	9.9×10^{-2}	7.1×10^{-2}	1.0×10^{-2}	8.3×10^{-2}	3.2×10^{-1}	8.8×10^{-2}
Pre-Olympics	35	100	1.3×10^{-1}	7.1×10^{-2}	2.3×10^{-2}	1.2×10^{-1}	3.2×10^{-1}	8.2×10^{-2}
During-Olympics	28	100	6.1×10^{-2}	4.0×10^{-2}	1.0×10^{-2}	5.1×10^{-2}	1.5×10^{-1}	5.7×10^{-2}
Post-Olympics	31	100	9.9×10^{-2}	7.8×10^{-2}	1.2×10^{-2}	7.6×10^{-2}	2.8×10^{-1}	9.4×10^{-2}
Th (μg/m^3)								
Whole period	94	98	7.8×10^{-5}	9.0×10^{-5}	8.3×10^{-9}	5.3×10^{-5}	5.0×10^{-4}	6.3×10^{-5}
Pre-Olympics	35	100	4.6×10^{-5}	5.9×10^{-5}	1.6×10^{-6}	3.3×10^{-5}	3.0×10^{-4}	3.3×10^{-5}
During-Olympics	28	93	7.1×10^{-5}	8.9×10^{-5}	8.3×10^{-9}	4.9×10^{-5}	4.7×10^{-4}	6.8×10^{-5}
Post-Olympics	31	100	1.2×10^{-4}	1.0×10^{-4}	2.9×10^{-5}	8.1×10^{-5}	5.0×10^{-4}	7.3×10^{-5}
U (μg/m^3)								
Whole period	94	100	4.8×10^{-5}	3.3×10^{-5}	6.5×10^{-6}	3.8×10^{-5}	2.0×10^{-4}	3.0×10^{-5}
Pre-Olympics	35	100	3.9×10^{-5}	1.6×10^{-5}	6.5×10^{-6}	3.7×10^{-5}	8.7×10^{-5}	1.7×10^{-5}
During-Olympics	28	100	4.3×10^{-5}	2.5×10^{-5}	9.0×10^{-6}	3.6×10^{-5}	9.7×10^{-5}	3.3×10^{-5}
Post-Olympics	31	100	6.2×10^{-5}	4.6×10^{-5}	1.5×10^{-5}	4.5×10^{-5}	2.0×10^{-4}	6.7×10^{-5}

[a] Summary statistics are based on 24-hour averages (from ~10 AM to ~10 AM next day).

APPENDIX B. Biomarker Level Summary Statistics Based on Simple Algebraic Calculations

Table B.1. Biomarkers of Autonomic Dysfunction[a]

Index / Period[b]	*n*	Mean	SD	Min	Median	Max	% BDL[c]
HR (bpm)							
Pre-Olympics	239	67	10	47	66	106	0.00
During-Olympics	234	66	9	44	66	93	0.00
Post-Olympics	247	66	9	45	65	97	0.00
HR Variability							
HF (ms^2)							
Pre-Olympics	235	859.8	789.6	8.1	611.8	4082.5	0.00
During-Olympics	233	805.2	695.3	28.3	574.2	4069.5	0.00
Post-Olympics	246	880.2	736.5	14.7	693.4	4617.9	0.00
LF (ms^2)							
Pre-Olympics	235	613.2	498.8	62.0	500.0	3476.4	0.00
During-Olympics	233	518.2	389.6	61.2	405.8	2590.1	0.00
Post-Olympics	246	529.8	430.5	7.3	414.5	3965.4	0.00
LF/HF							
Pre-Olympics	235	1.2	1.2	0.1	0.8	8.8	0.00
During-Olympics	233	1.0	0.9	0.1	0.7	6.5	0.00
Post-Olympics	245	0.9	1.0	0.1	0.6	7.2	0.00
rMSSD (ms)							
Pre-Olympics	239	70	66	6	53	568	0.00
During-Olympics	235	68	65	7	52	658	0.00
Post-Olympics	247	63	58	16	50	651	0.00
SDNN (ms)							
Pre-Olympics	239	68	40	21	58	311	0.00
During-Olympics	235	68	52	22	56	542	0.00
Post-Olympics	247	65	38	20	54	349	0.00
VLF (ms^2)							
Pre-Olympics	235	841.9	637.3	73.4	699.8	5324.1	0.00
During-Olympics	233	764.5	630.1	84.2	591.9	4908.3	0.00
Post-Olympics	246	874.5	730.7	28.5	664.6	4910.8	0.00
Total power (ms^2)							
Pre-Olympics	235	2383.5	1390.6	206.8	2123.6	6845.4	0.00
During-Olympics	233	2155.0	1250.3	440.1	1835.6	6755.4	0.00
Post-Olympics	246	2343.7	1369.1	52.0	2014.0	8335.6	0.00
Blood Pressure							
DBP (mmHg)							
Pre-Olympics	248	61	6.6	44	60	80	0.00
During-Olympics	249	60	6.8	39	59	81	0.00
Post-Olympics	247	62	7.8	42	61	88	0.00
SBP (mmHg)							
Pre-Olympics	248	105	10.0	79	105	134	0.00
During-Olympics	249	103	10.2	75	103	136	0.00
Post-Olympics	247	107	10.8	82	105	140	0.00

[a] Data were not adjusted for repeated-measure structure or covariates.

[b] Pre-Olympic period: 06/10 to 07/07/2008; during-Olympic period: 08/04 to 08/29/2008; and post-Olympic period: 10/06 to 10/30/2008.

[c] BDL indicates below detection limit.

Table B.2. Biomarkers of Systemic Inflammation and Oxidative Stress[a]

Biomarker / Period[b]	*n*	Mean	SD	Min	Median	Max	% BDL[c]
Plasma							
CRP							
Pre-Olympics	247	0.73	1.58	0.15	0.30	11.90	46.15
During-Olympics	243	0.58	1.13	0.15	0.15	12.40	51.85
Post-Olympics	241	0.63	1.38	0.15	0.15	12.40	58.51
Fibrinogen							
Pre-Olympics	248	2.48	0.44	1.17	2.44	4.95	0.00
During-Olympics	249	2.41	0.37	1.13	2.38	3.46	0.00
Post-Olympics	247	2.86	0.56	1.05	2.80	5.15	0.00
Cell Counts							
RBCs ($\times 10^{12}$/L)							
Pre-Olympics	248	4.59	0.51	3.51	4.53	6.55	0.00
During-Olympics	249	4.60	0.50	3.53	4.56	6.01	0.00
Post-Olympics	247	4.48	0.50	3.44	4.40	5.92	0.00
WBCs ($\times 10^{9}$/L)							
Pre-Olympics	248	5.30	1.40	2.70	5.10	11.60	0.00
During-Olympics	249	5.32	1.29	2.60	5.20	10.90	0.00
Post-Olympics	247	5.13	1.31	2.80	4.90	10.20	0.00
Lymphocytes ($\times 10^{9}$/L)							
Pre-Olympics	248	1.67	0.41	0.80	1.60	2.86	0.00
During-Olympics	249	1.72	0.44	0.60	1.70	3.50	0.00
Post-Olympics	247	1.57	0.38	0.70	1.50	3.30	0.00
Lymphocytes (%)							
Pre-Olympics	248	32.46	7.71	12.00	32.75	54.30	0.00
During-Olympics	249	33.07	7.20	9.90	33.50	52.00	0.00
Post-Olympics	247	31.38	6.90	11.80	31.50	50.70	0.00
Neutrophils ($\times 10^{9}$/L)							
Pre-Olympics	248	3.13	1.22	1.04	2.88	9.44	0.00
During-Olympics	249	3.12	1.06	1.13	2.88	6.84	0.00
Post-Olympics	247	3.13	1.10	1.31	2.85	7.00	0.00
Neutrophils (%)							
Pre-Olympics	248	57.89	8.62	36.80	57.60	81.40	0.00
During-Olympics	249	57.69	8.16	37.20	57.70	82.10	0.00
Post-Olympics	247	59.94	7.54	40.40	59.70	81.20	0.00
Urine							
8-OHdG (mg/mol creatinine)							
Pre-Olympics	248	9.6	21.8	0.02	4.1	292.7	8.87
During-Olympics	244	7.7	18.5	0.02	3.1	237.2	18.44
Post-Olympics	247	8.2	14.9	0.01	3.9	145.5	10.93

[a] Data were not adjusted for repeated-measure structure or covariates.

[b] Pre-Olympic period: 06/10 to 07/07/2008; during-Olympic period: 08/04 to 08/29/2008; and post-Olympic period: 10/06 to 10/30/2008.

[c] Concentrations below the detection limit (BDL) were designated as half of the detection limit in the calculations.

Table B.3. Biomarkers of Pulmonary Inflammation and Oxidative Stress[a]

Biomarker / Period[b]	*n*	Mean	SD	Min	Median	Max	% BDL[c]
FeNO (ppb)							
Pre-Olympics	248	13.14	6.40	0.60	11.65	44.90	0.00
During-Olympics	249	7.26	5.24	0.17	6.04	38.04	0.00
Post-Olympics	246	14.22	8.12	3.00	12.45	54.80	0.00
EBC nitrite (µM)							
Pre-Olympics	248	8.51	5.42	1.84	6.96	36.47	0.00
During-Olympics	248	5.33	3.10	0.08	4.29	19.69	2.02
Post-Olympics	198	5.11	2.40	1.86	4.71	23.61	0.00
EBC nitrate (µM)							
Pre-Olympics	248	3.05	1.43	1.13	2.67	11.07	0.00
During-Olympics	248	2.92	1.67	0.91	2.54	12.74	0.00
Post-Olympics	198	6.63	5.78	0.36	5.20	32.13	0.00
EBC nitrate+nitrite (µM)							
Pre-Olympics	248	11.56	5.84	4.18	9.74	41.60	0.00
During-Olympics	248	8.24	3.35	3.06	7.56	23.67	0.00
Post-Olympics	198	11.74	5.96	3.33	11.06	37.18	0.00
EBC pH							
Pre-Olympics	247	7.43	0.38	5.85	7.52	8.11	0.00
During-Olympics	249	7.46	0.55	4.50	7.55	8.21	0.00
Post-Olympics	247	7.61	0.29	6.57	7.64	8.23	0.00
EBC 8-isoprostane (pg/mL)							
Pre-Olympics	246	4.33	3.25	0.78	4.02	11.64	31.71
During-Olympics	246	2.54	2.97	0.78	0.78	15.40	55.69
Post-Olympics	244	4.66	3.53	0.78	4.65	16.03	26.23

[a] Data were not adjusted for repeated-measure structure or covariates.

[b] Pre-Olympic period: 06/10 to 07/07/2008; during-Olympic period: 08/04 to 08/29/2008; and post-Olympic period: 10/06 to 10/30/2008.

[c] Concentrations below the detection limit (BDL) were designated as half of the detection limit in the calculations.

Table B.4. Biomarkers of Hemostasis[a]

Biomarker / Period[b]	*n*	Mean	SD	Min	Median	Max	% BDL
sCD40L (ng/mL)							
Pre-Olympics	246	1.93	0.49	0.61	1.89	3.10	0.00
During-Olympics	246	1.80	0.47	0.72	1.79	4.01	0.00
Post-Olympics	244	2.00	0.67	0.65	1.99	7.54	0.00
sCD62p (ng/mL)							
Pre-Olympics	246	6.68	1.58	3.02	6.50	10.53	0.00
During-Olympics	246	5.20	1.54	3.09	4.83	11.63	0.00
Post-Olympics	244	5.45	1.20	2.92	5.28	10.53	0.00
Platelet aggregation (% platelets aggregated)							
Pre-Olympics	248	58.57	21.16	5.22	67.47	98.00	0.00
During-Olympics	249	63.33	17.71	10.84	68.85	98.28	0.00
Post-Olympics	246	57.93	22.17	5.66	66.99	98.50	0.00
vWF (%)							
Pre-Olympics	247	102.24	30.01	25.42	101.34	176.70	0.00
During-Olympics	249	89.91	29.89	7.21	90.51	174.93	0.00
Post-Olympics	247	83.85	28.06	25.10	82.80	165.57	0.00

[a] Data were not adjusted for repeated-measure structure or covariates.

[b] Pre-Olympic period: 06/10 to 07/07/2008; during-Olympic period: 08/04 to 08/29/2008; and post-Olympic period: 10/06 to 10/30/2008.

APPENDIX C. Pollutant–Biomarker Association Results from Two-Pollutant Models

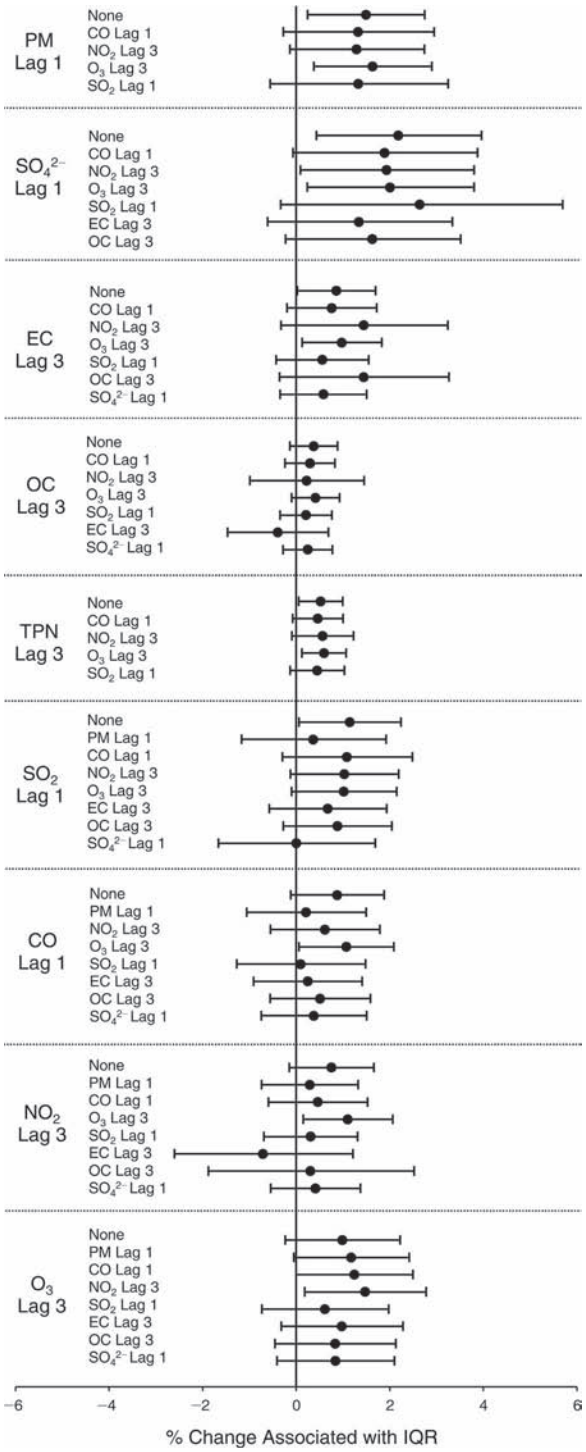


Figure C.1. Estimated means and 95% CIs for the percent change in HR associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), sex, day of the week, and a second pollutant.

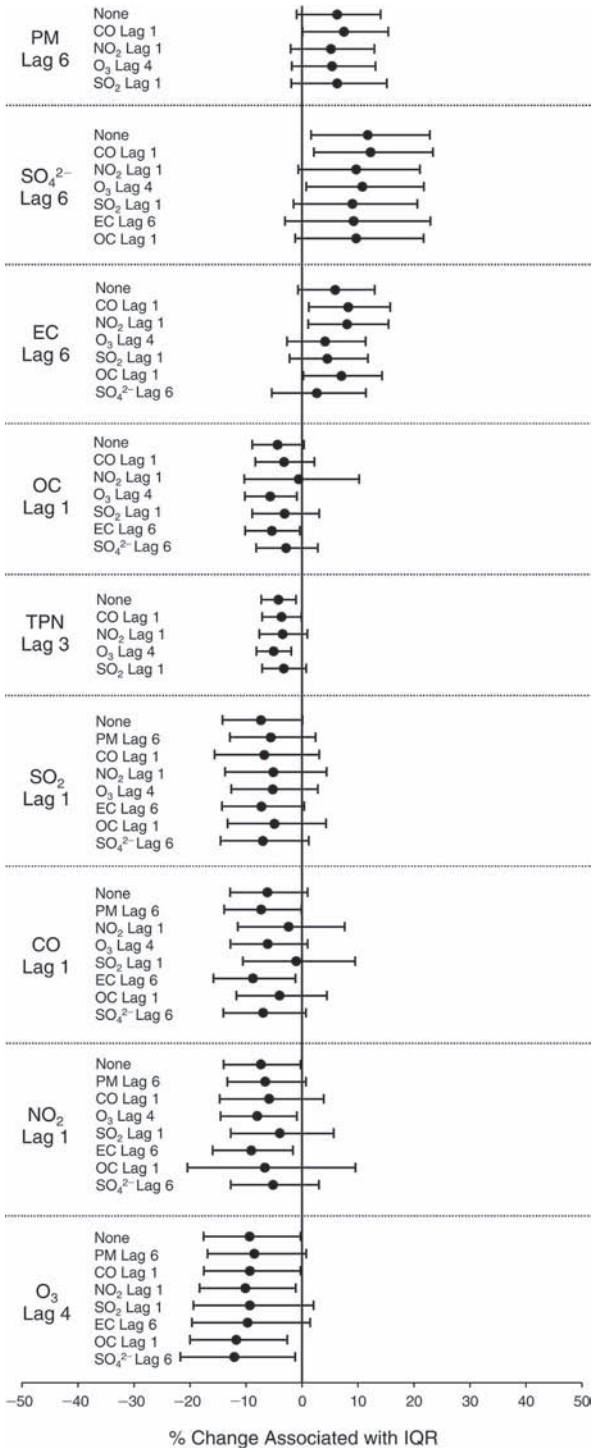


Figure C.2. Estimated means and 95% CIs for the percent change in HF (HRV) associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 7-day moving average of temperature (df = 1), sex, day of the week, and a second pollutant.

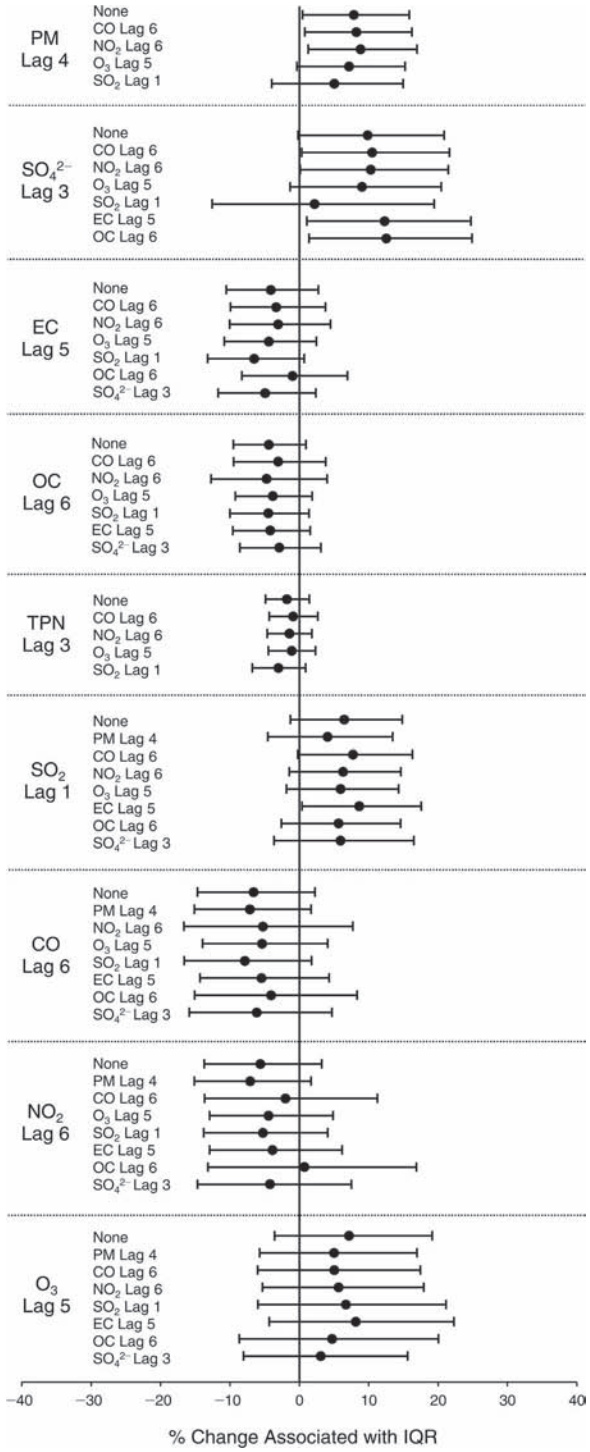


Figure C.3. Estimated means and 95% CIs for the percent change in LF (HRV) associated with one IQR increase in pollutant concentration, controlling for temperature (df = 2), RH (df = 1), 7-day moving average of temperature (df = 1), 5-day moving average of RH (df = 1), sex, day of the week, and a second pollutant.

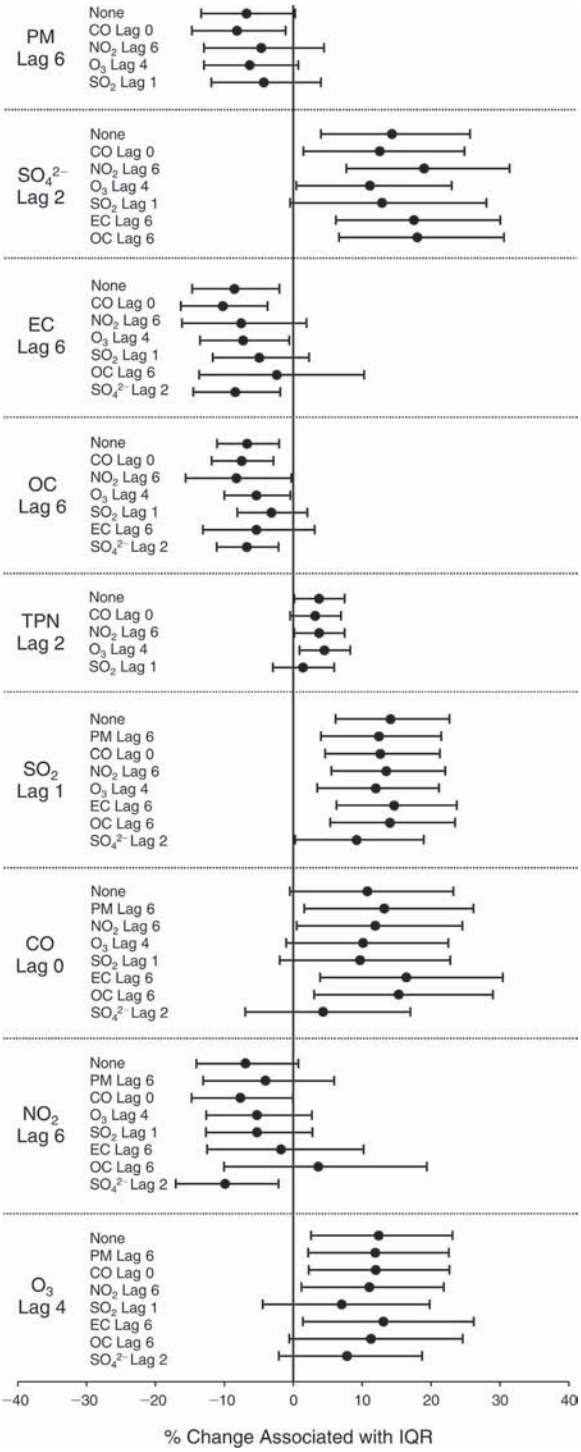


Figure C.4. Estimated means and 95% CIs for the percent change in LF/HF (HRV) associated with one IQR increase in pollutant concentration, controlling for temperature (df = 2), RH (df = 1), 7-day moving average of temperature (df = 1), 2-day moving average of RH (df = 1), sex, day of the week, and a second pollutant.

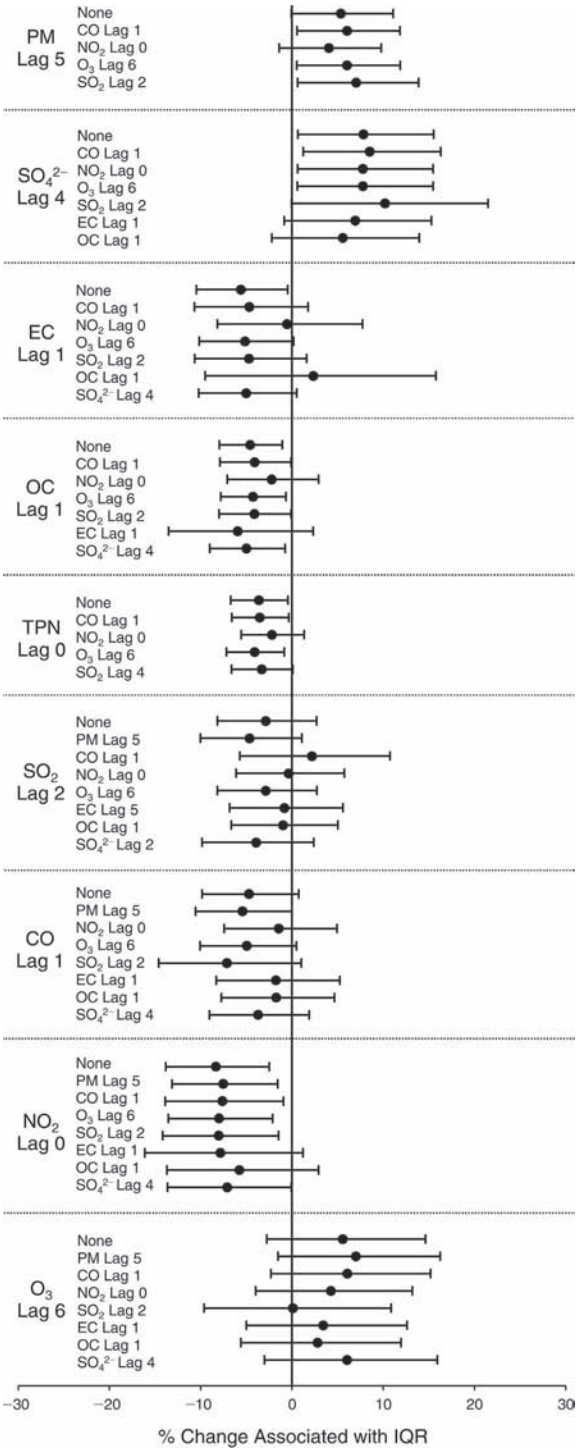


Figure C.5. Estimated means and 95% CIs for the percent change in rMSSD (HRV) associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 7-day moving average of temperature (df = 1), sex, day of the week, and a second pollutant.

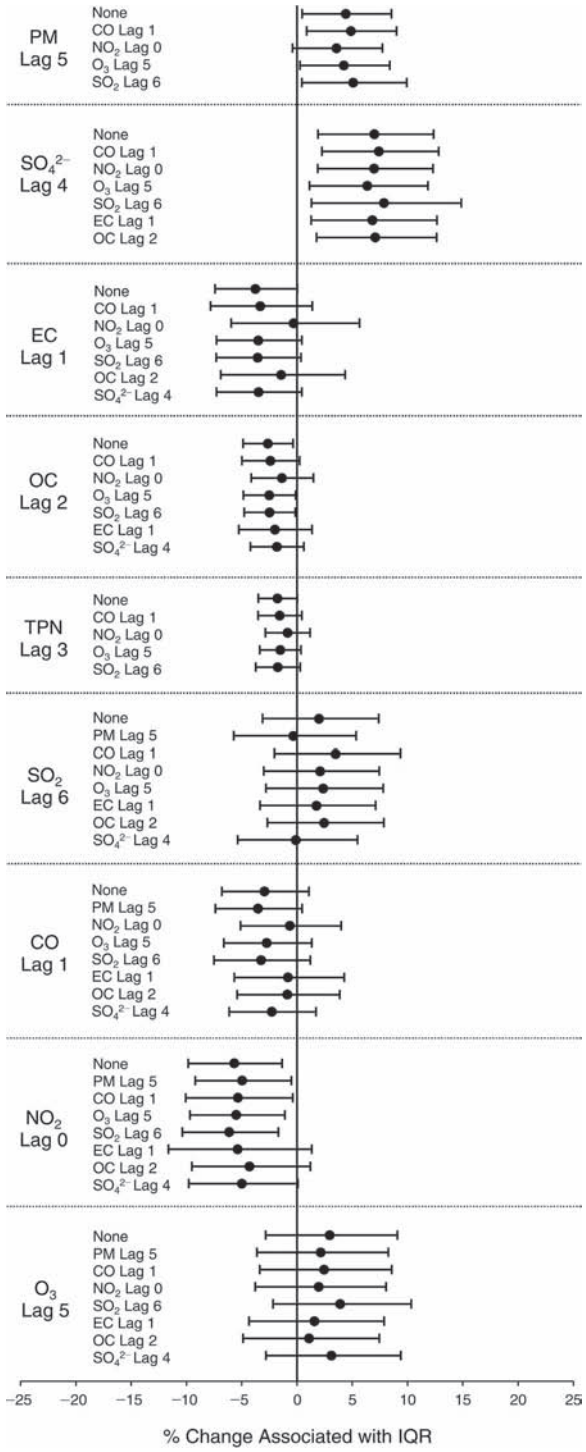


Figure C.6. Estimated means and 95% CIs for the percent change in SDNN (HRV) associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 7-day moving average of temperature (df = 1), sex, day of the week, and a second pollutant.

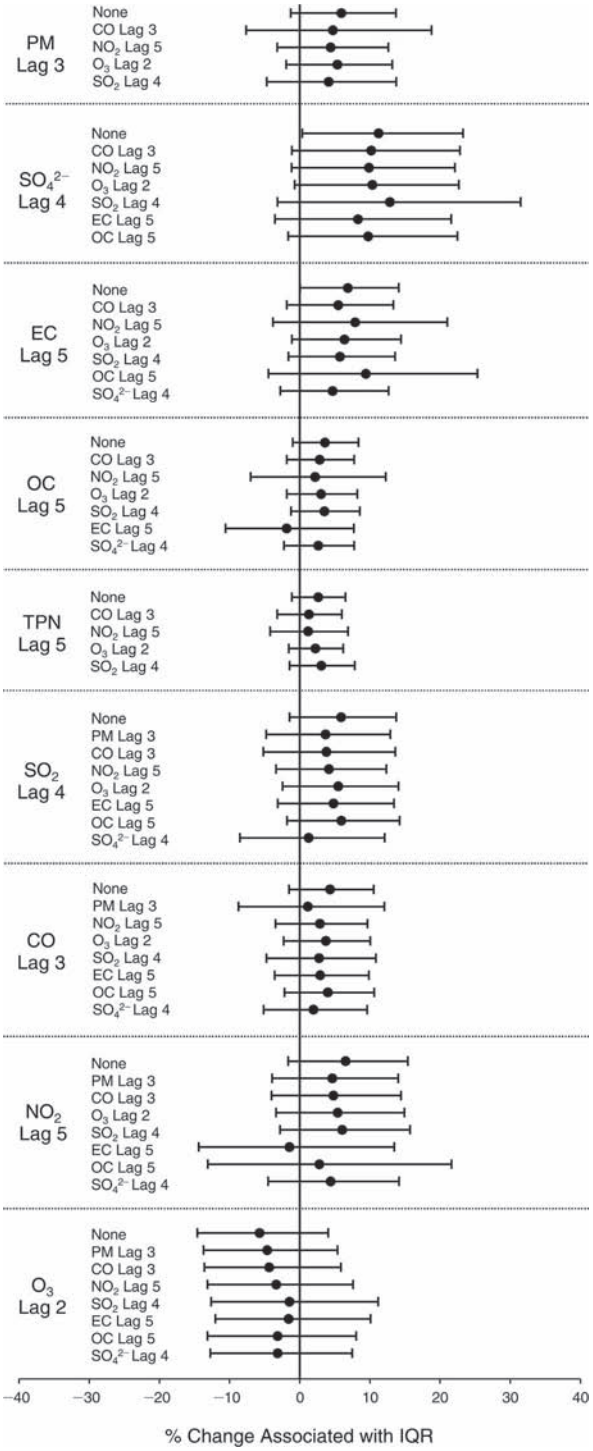


Figure C.7. Estimated means and 95% CIs for the percent change in VLF (HRV) associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 7-day moving average of temperature (df = 1), sex, day of the week, and a second pollutant.

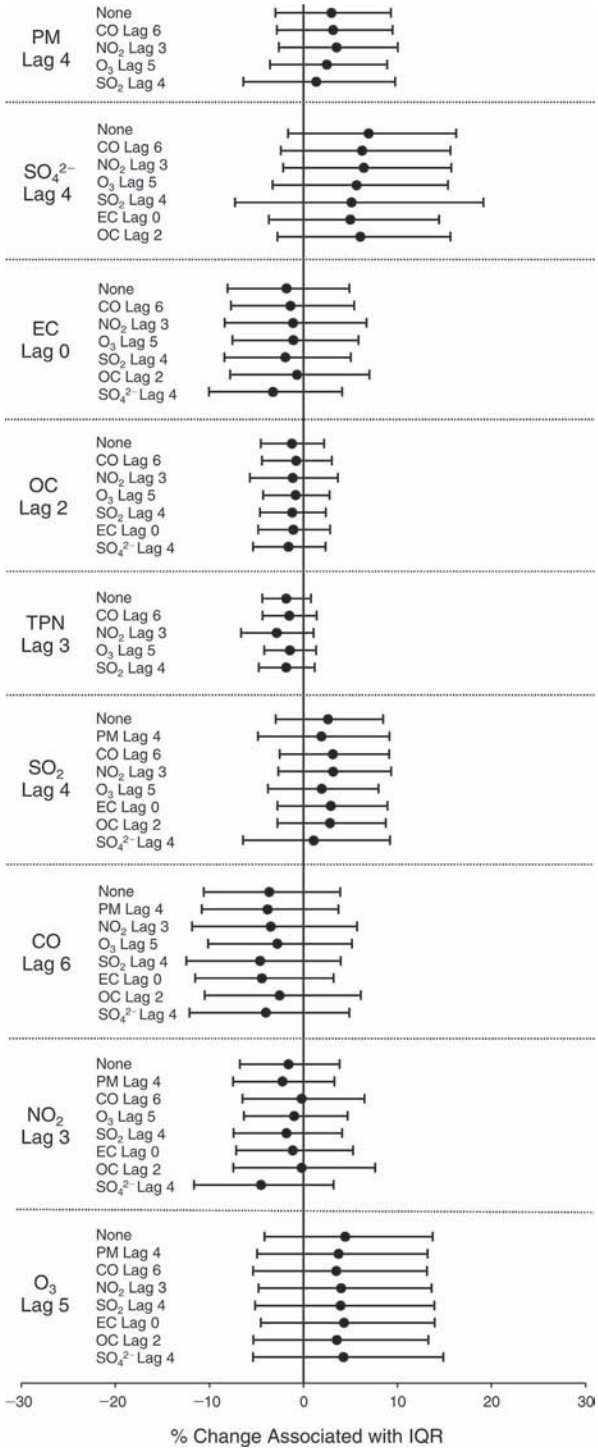


Figure C.8. Estimated means and 95% CIs for the percent change in total power (HRV) associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 7-day moving average of temperature (df = 1), 5-day moving average of RH (df = 1), sex, day of the week, and a second pollutant.

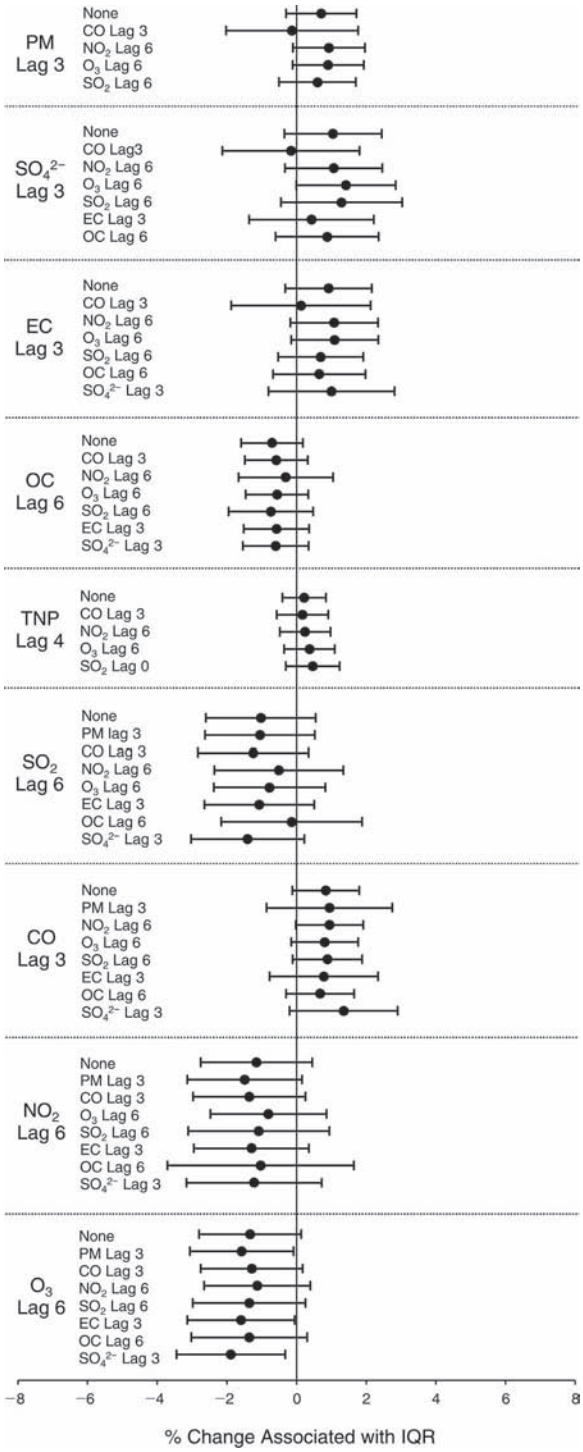


Figure C.9. Estimated means and 95% CIs for the percent change in DBP associated with one IQR increase in pollutant concentration, controlling for temperature (df = 3), RH (df = 3), 7-day moving average of temperature (df = 3), 5-day moving average of RH (df = 3), sex, day of the week, and a second pollutant.

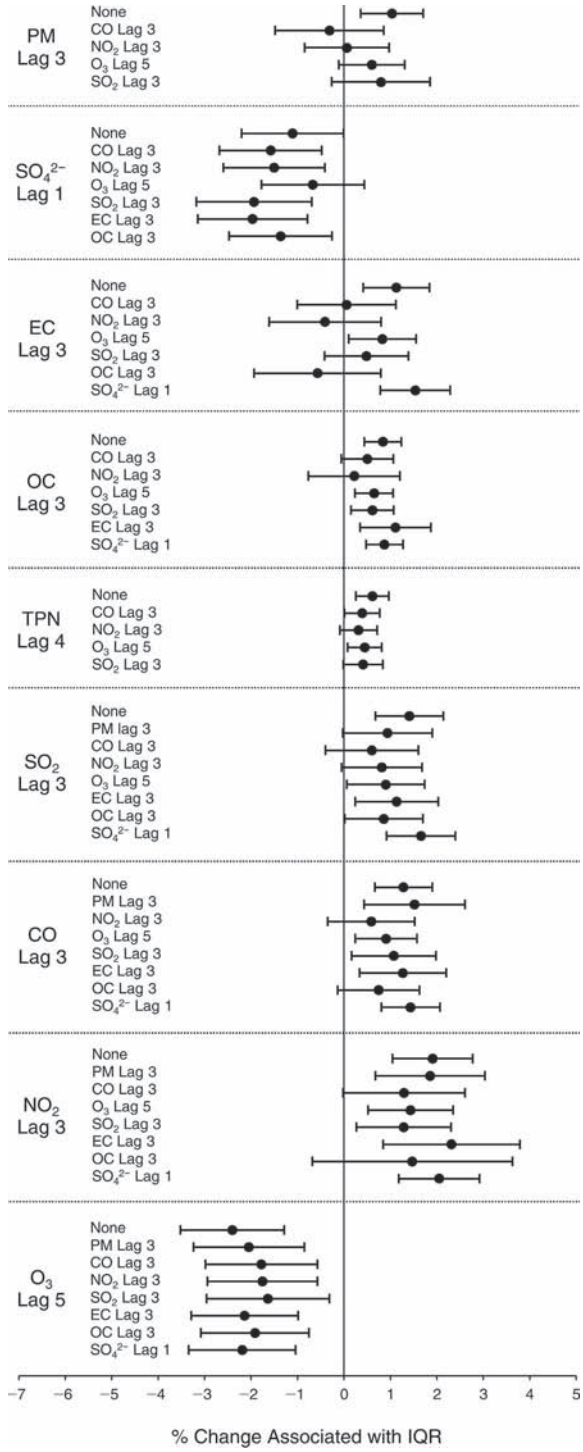


Figure C.10. Estimated means and 95% CIs for the percent change in SBP associated with one IQR increase in pollutant concentration, controlling for temperature (df = 3), RH (df = 2), 7-day moving average of temperature (df = 3), 2-day moving average of RH (df = 3), sex, day of the week, and a second pollutant.

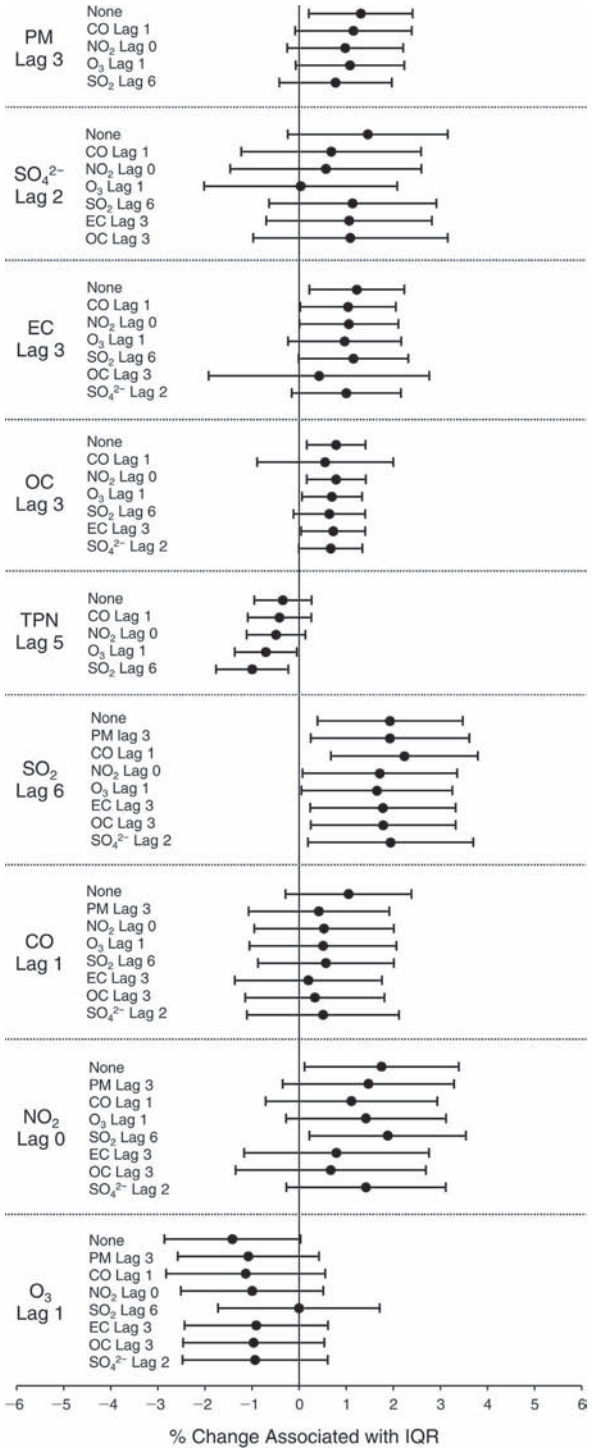


Figure C.11. Estimated means and 95% CIs for the percent change in fibrinogen level associated with one IQR increase in pollutant concentration, controlling for temperature (df = 3), RH (df = 1), 6-day moving average of temperature (df = 1), sex, day of the week, and a second pollutant.

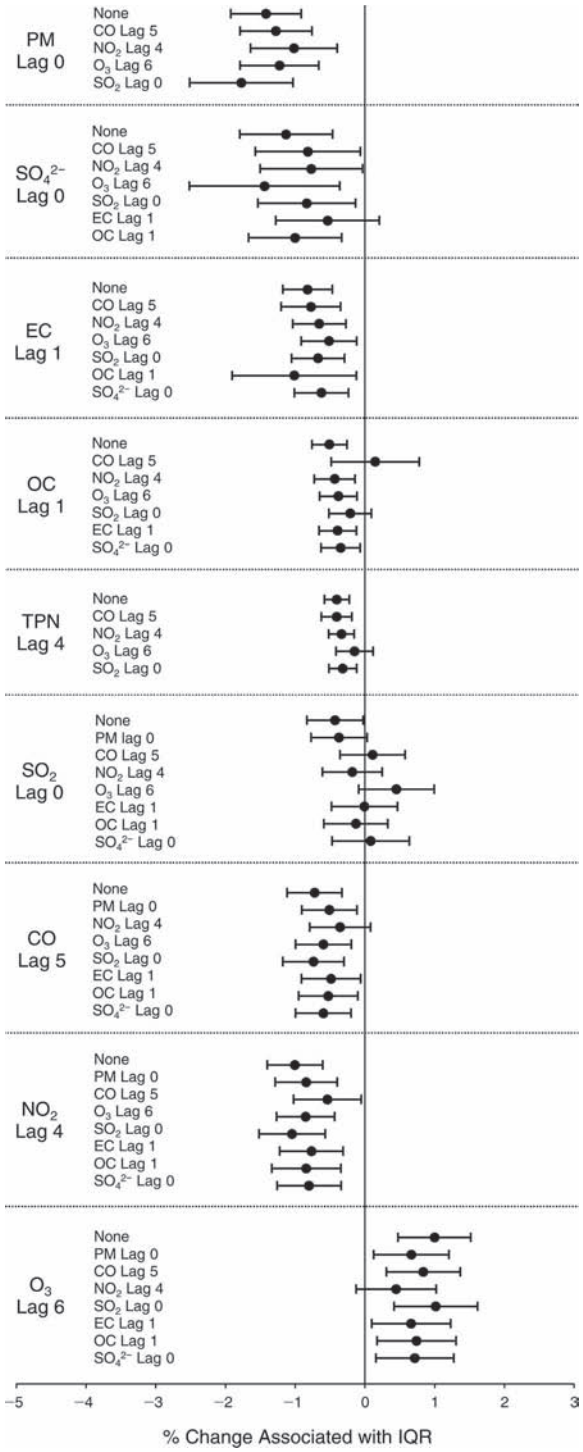


Figure C.12. Estimated means and 95% CIs for the percent change in RBC count associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 4-day moving average of RH (df = 1), sex, day of the week, and a second pollutant.

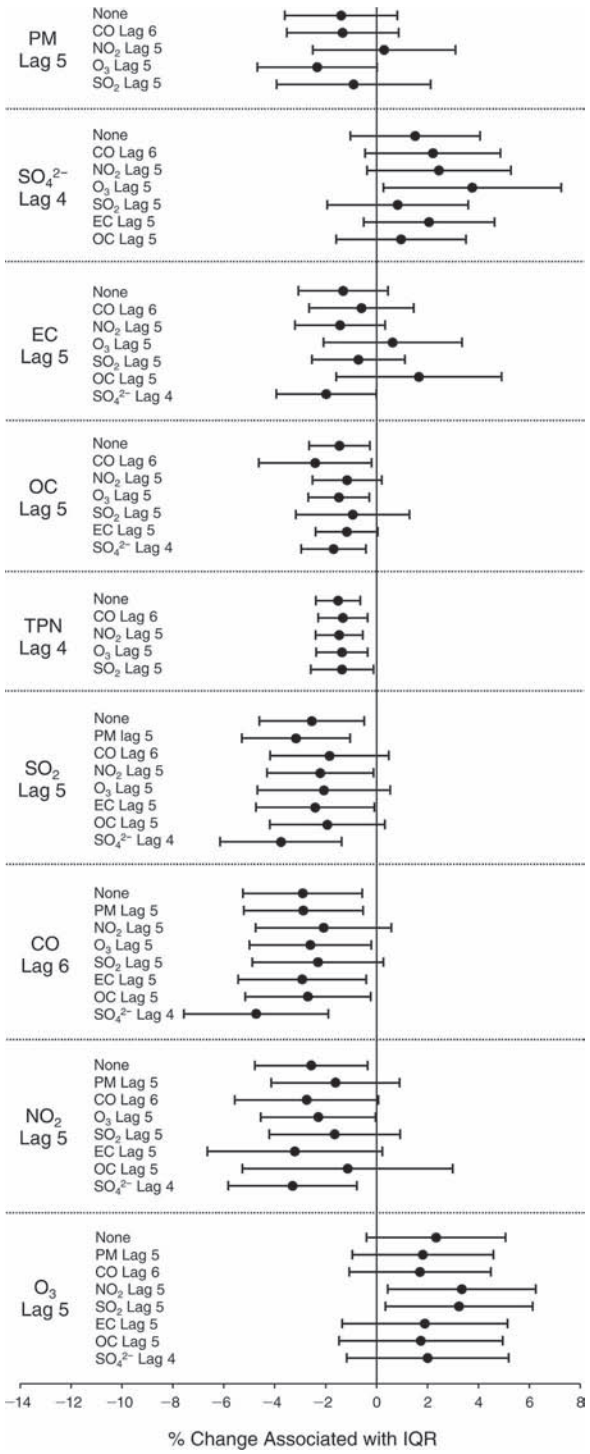


Figure C.13. Estimated means and 95% CIs for the percent change in WBC count associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 7-day moving average of temperature (df = 1), 7-day moving average of RH (df = 2), sex, day of the week, and a second pollutant.

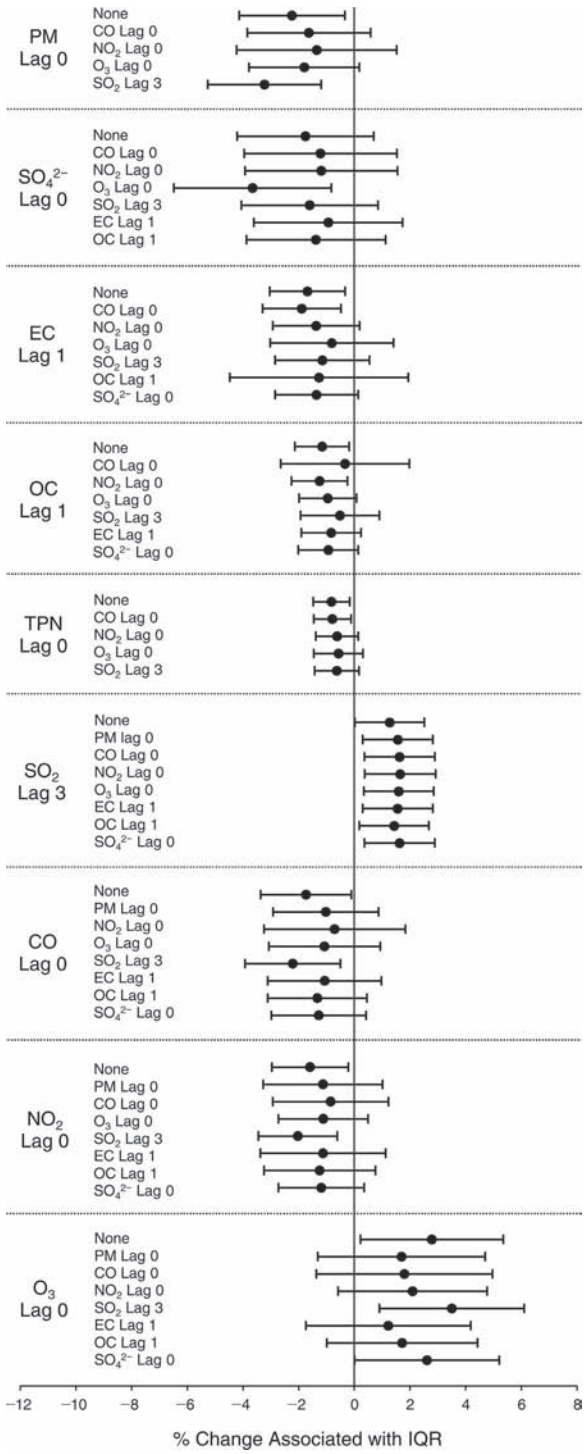


Figure C.14. Estimated means and 95% CIs for the percent change in lymphocyte count associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), sex, day of the week, and a second pollutant.

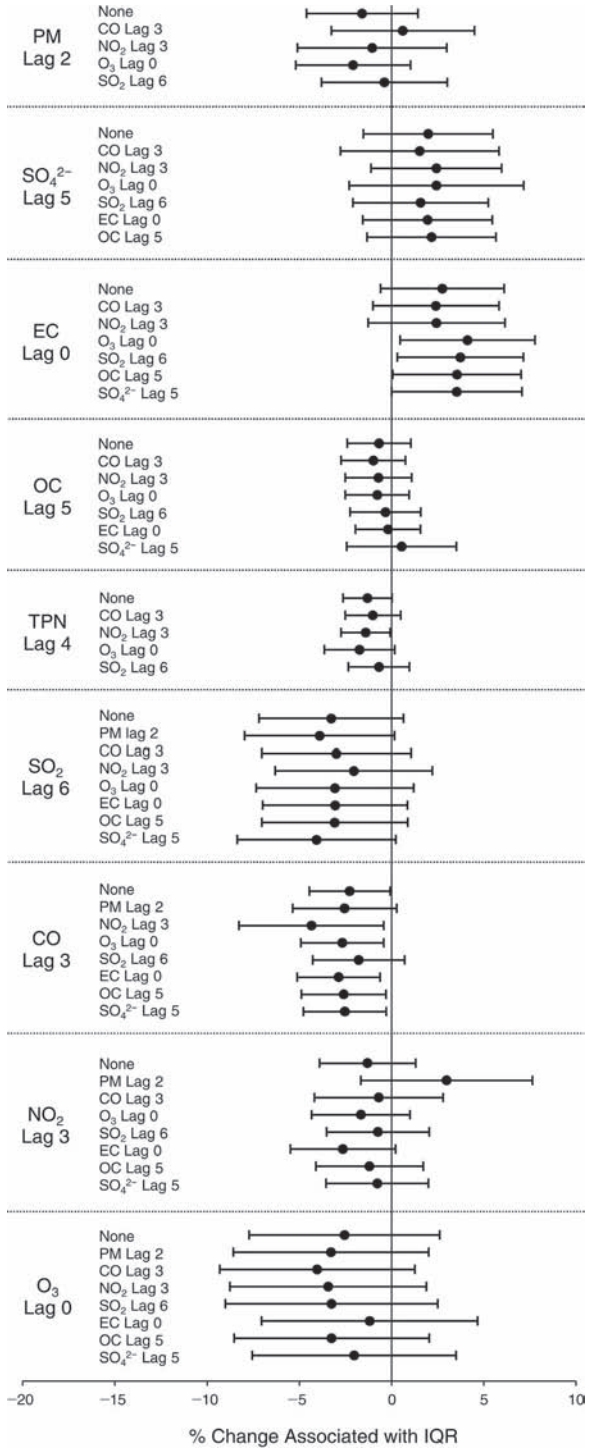


Figure C.15. Estimated means and 95% CIs for the percent change in neutrophil count associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 7-day moving average of temperature (df = 1), sex, day of the week, and a second pollutant.

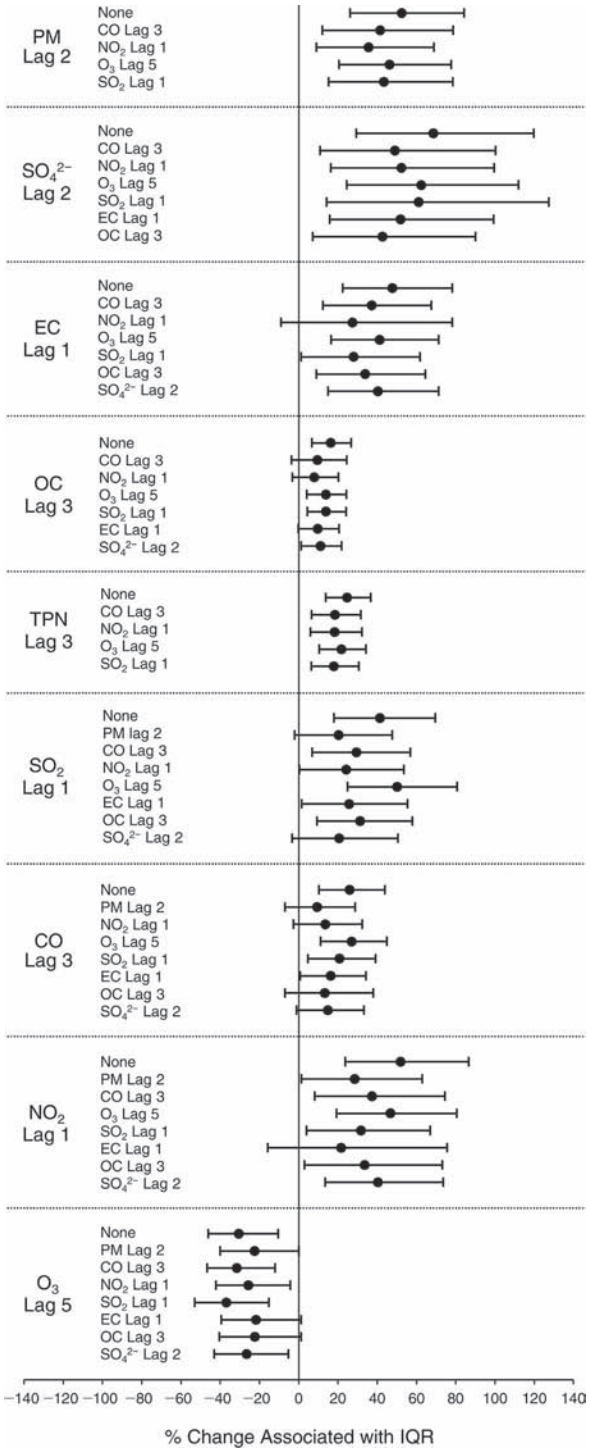


Figure C.16. Estimated means and 95% CIs for the percent change in urinary 8-OHdG (corrected by creatinine) associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 7-day moving average of temperature (df = 1), 2-day moving average of RH (df = 3), sex, day of the week, and a second pollutant.

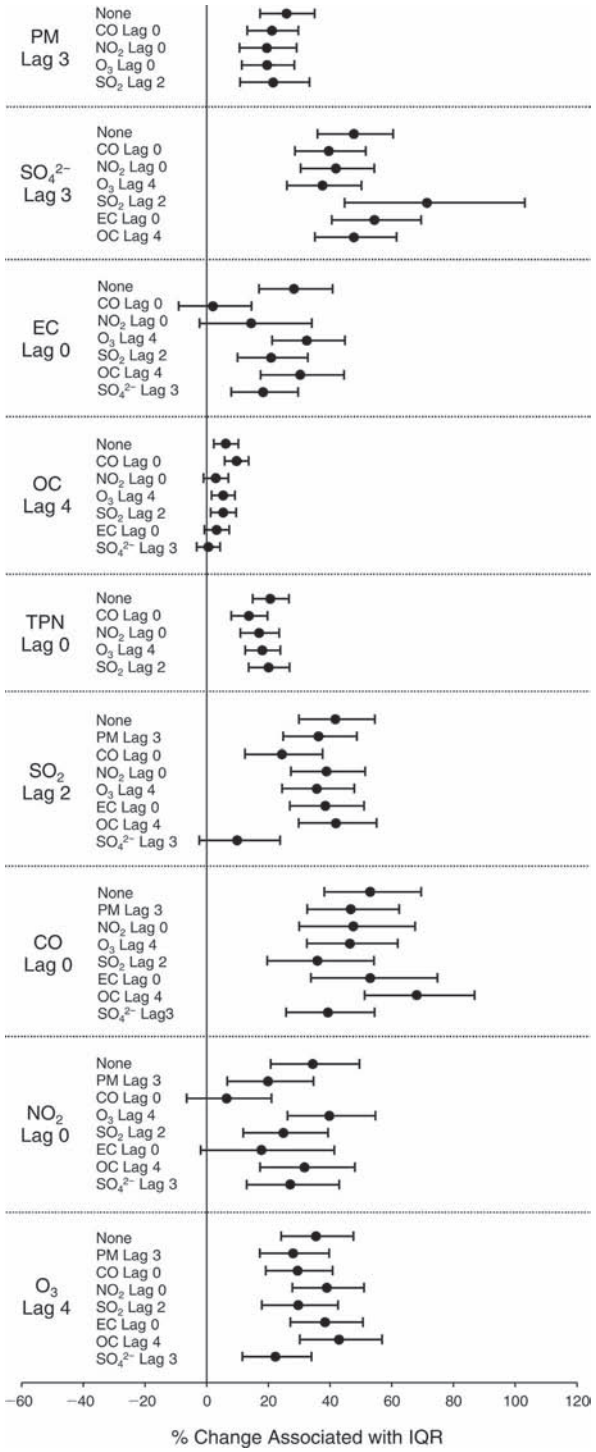


Figure C.17. Estimated means and 95% CIs for the percent change in FeNO level associated with one IQR increase in pollutant concentration, controlling for temperature (df = 2), RH (df = 3), 7-day moving average of temperature (df = 2), 7-day moving average of RH (df = 3), sex, day of the week, and a second pollutant.

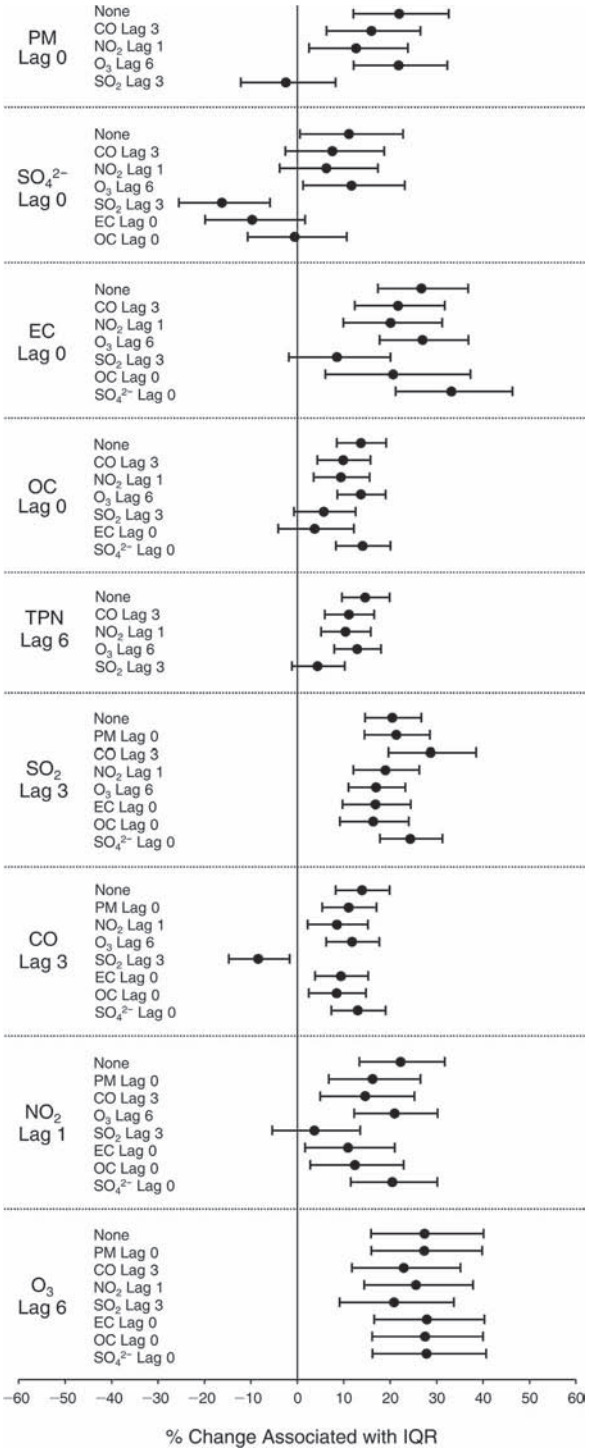


Figure C.18. Estimated means and 95% CIs for the percent change in EBC nitrite level associated with one IQR increase in pollutant concentration, controlling for temperature (df = 2), RH (df = 1), 7-day moving average of temperature (df = 3), 3-day moving average of RH (df = 3), sex, day of the week, and a second pollutant.

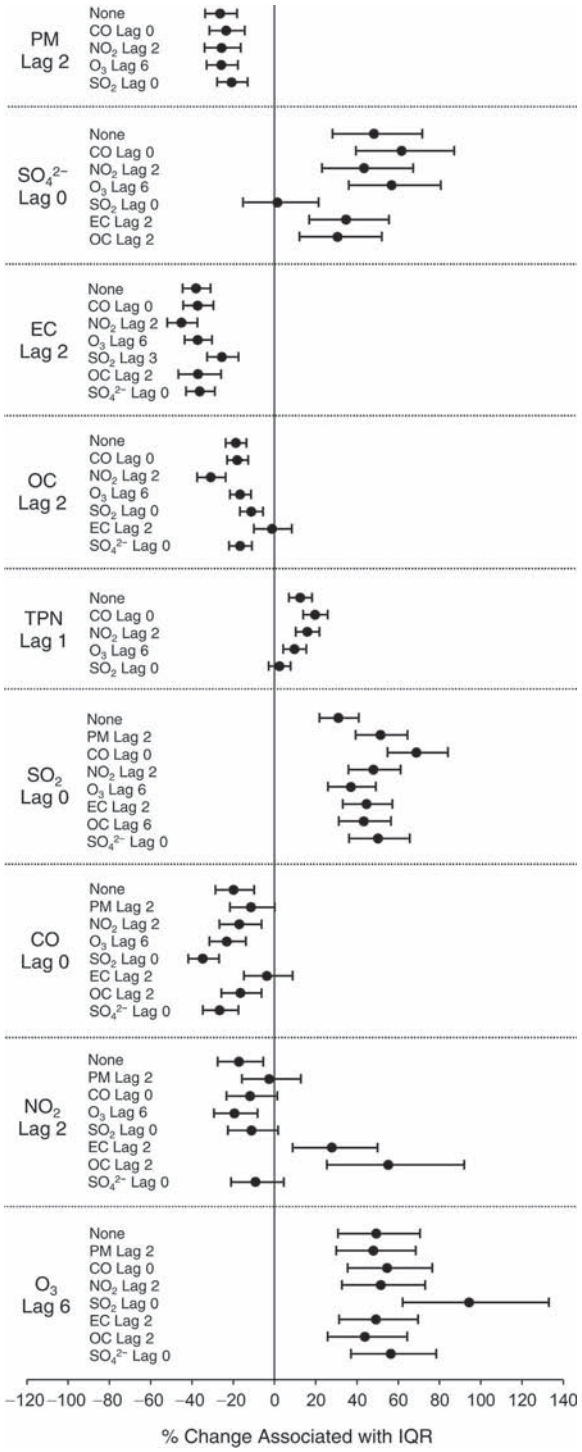


Figure C.19. Estimated means and 95% CIs for the percent change in EBC nitrate level associated with one IQR increase in pollutant concentration, controlling for temperature (df = 2), RH (df = 1), 7-day moving average of temperature (df = 3), 3-day moving average of RH (df = 3), sex, day of the week, and a second pollutant.

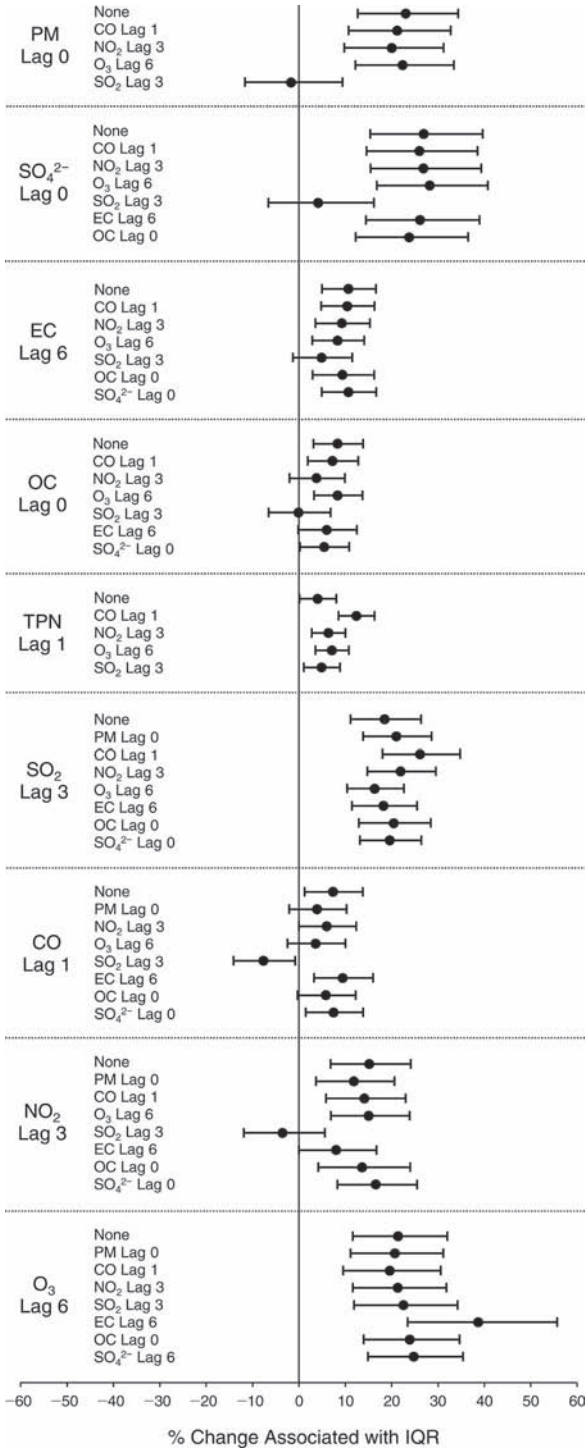


Figure C.20. Estimated means and 95% CIs for the percent change in EBC nitrite+nitrate level associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 3), 7-day moving average of temperature (df = 3), 5-day moving average of RH (df = 3), sex, day of the week, and a second pollutant

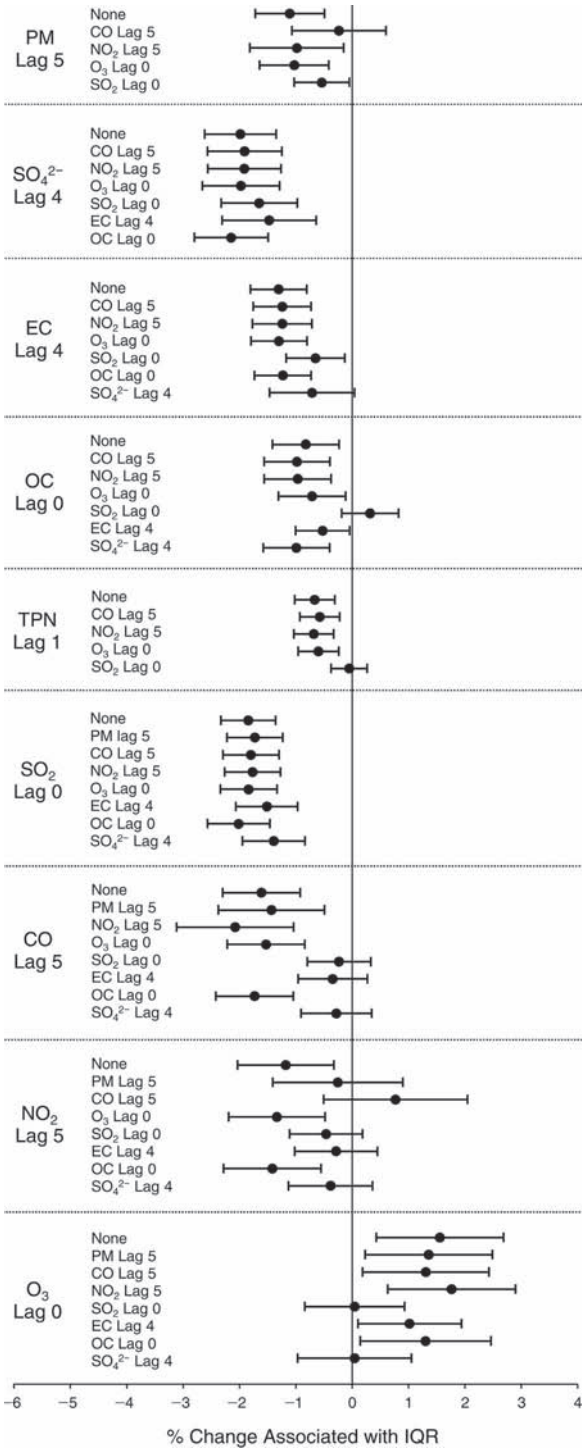


Figure C.21. Estimated means and 95% CIs for the percent change in EBC pH level associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 6-day moving average of temperature (df = 3), 3-day moving average of RH (df = 1), sex, day of the week, and a second pollutant.

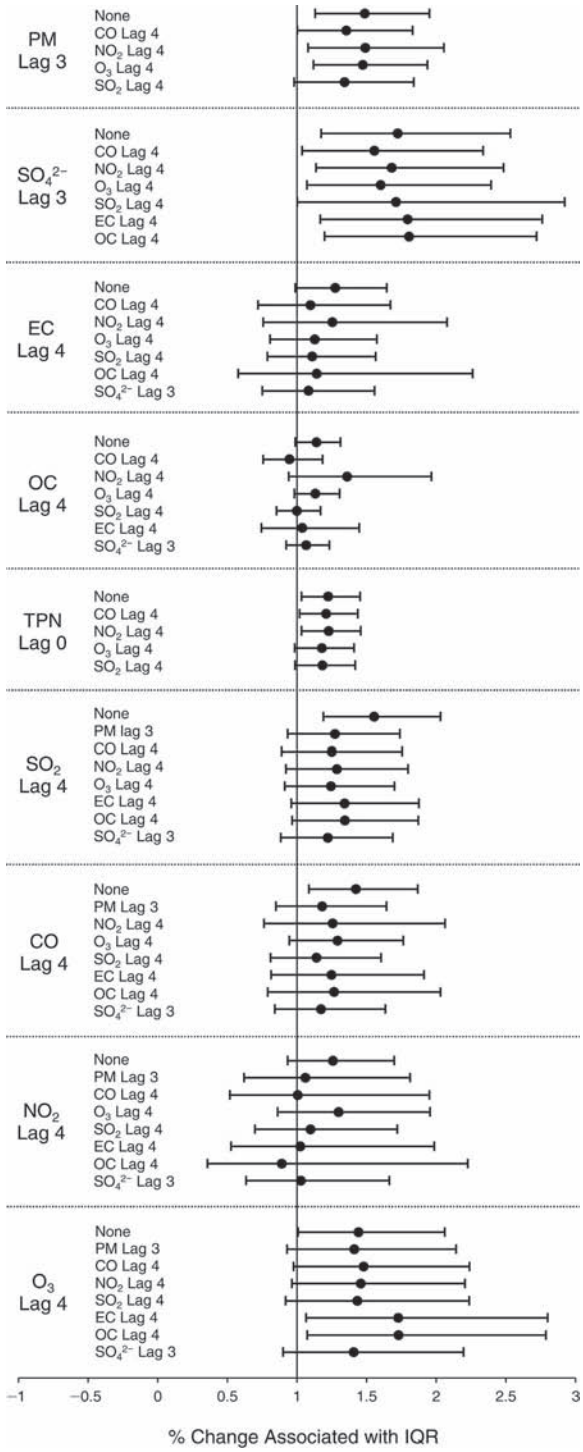


Figure C.22. Estimated means and 95% CIs for the percent change in odds for getting a greater-than-75th-percentile value of EBC 8-isoprostane associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 7-day moving average of temperature (df = 2), 2-day moving average of RH (df = 1), sex, day of the week, and a second pollutant.

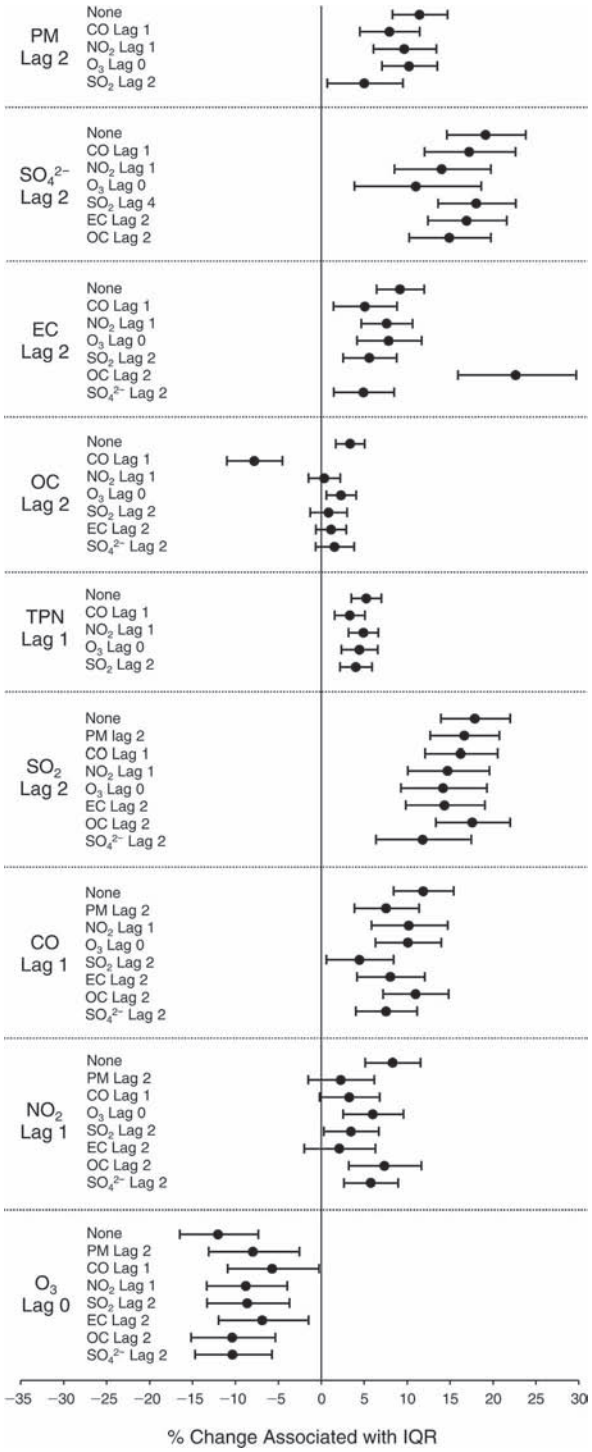


Figure C.23. Estimated means and 95% CIs for the percent change in sCD62P level associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 3), 7-day moving average of temperature (df = 2), 4-day moving average of RH (df = 2), sex, day of the week, and a second pollutant.

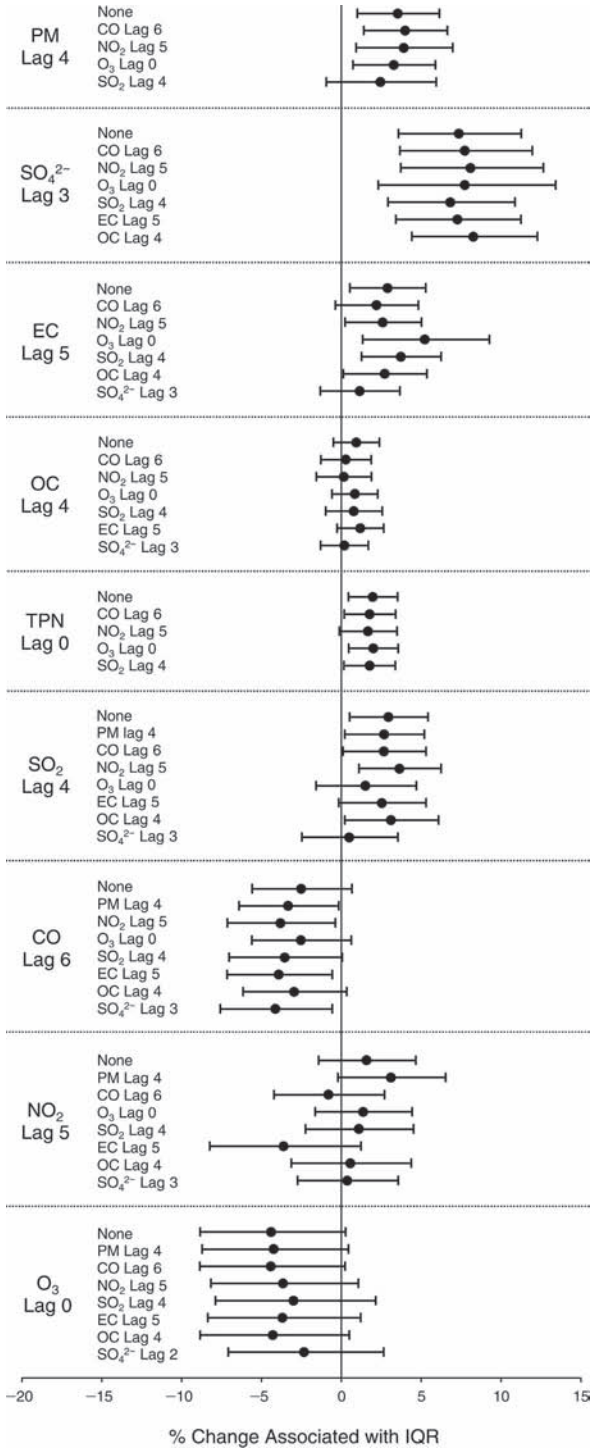


Figure C.24. Estimated means and 95% CIs for the percent change in sCD40L level associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 5-day moving average of temperature (df = 1), 2-day moving average of RH (df = 1), sex, day of the week, and a second pollutant.

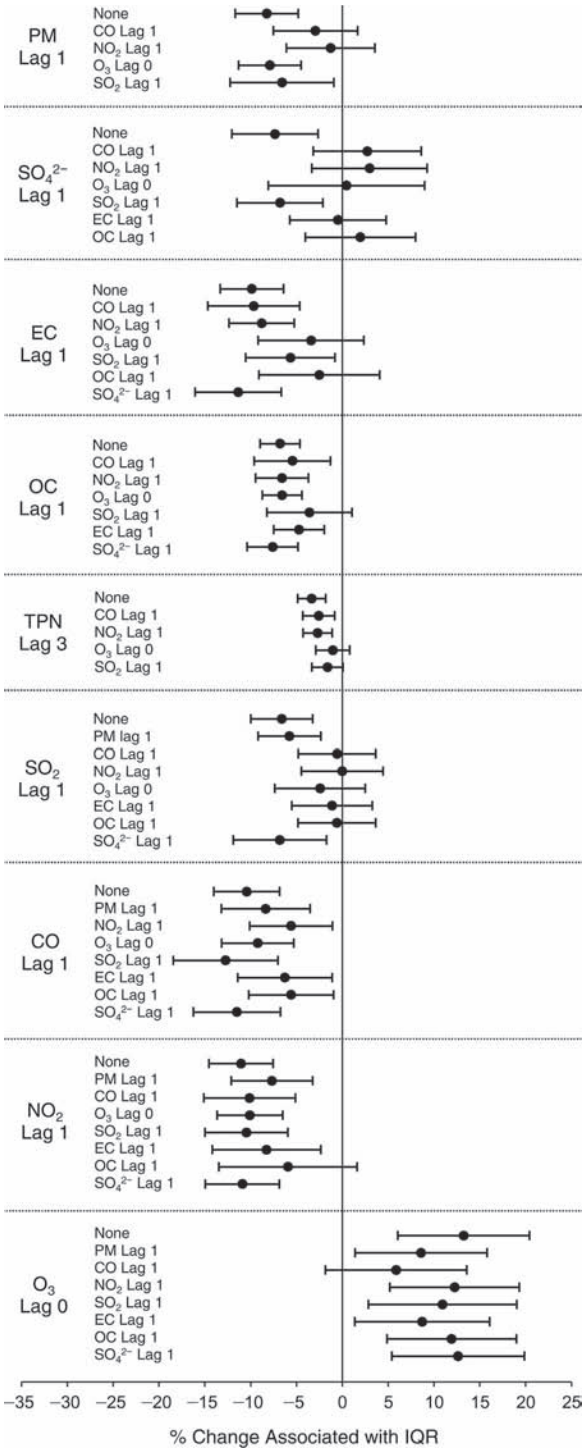


Figure C.25. Estimated means and 95% CIs for the percent change in platelet aggregation level associated with one IQR increase in pollutant concentration, controlling for temperature (df = 3), RH (df = 3), 7-day moving average of temperature (df = 3), 3-day moving average of RH (df = 3), sex, day of the week, and a second pollutant.

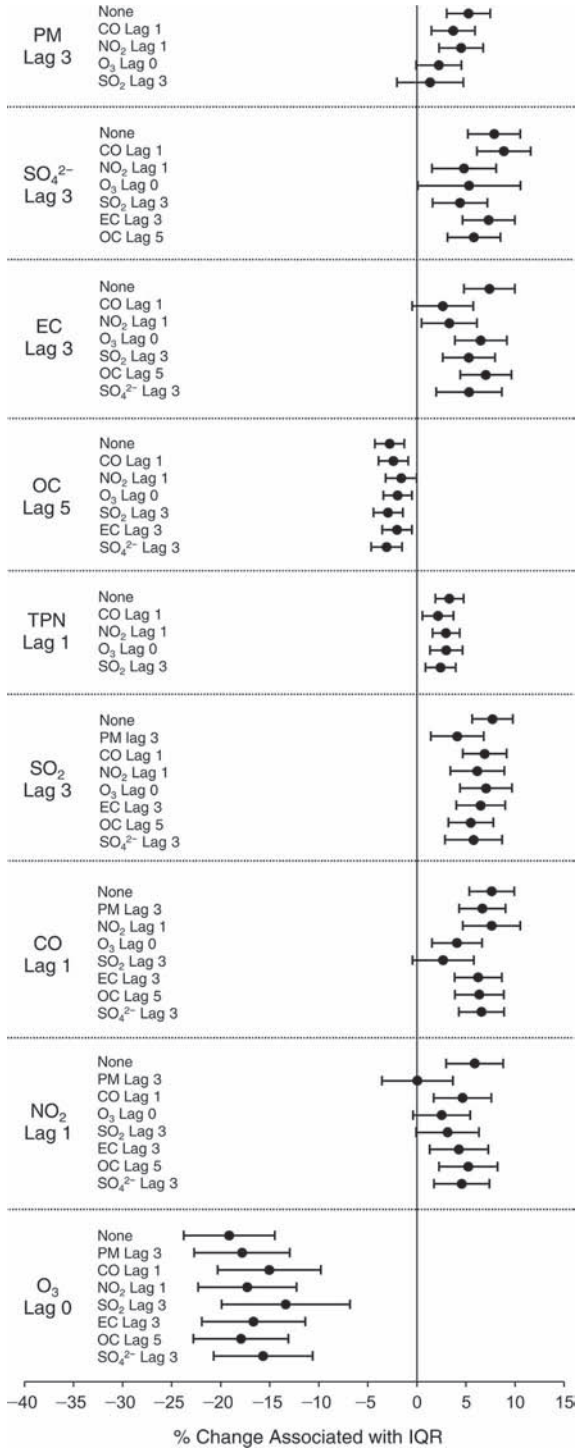


Figure C.26. Estimated means and 95% CIs for the percent change in vWF level associated with one IQR increase in pollutant concentration, controlling for temperature (df = 3), RH (df = 3), 6-day moving average of temperature (df = 3), 6-day moving average of RH (df = 3), sex, day of the week, and a second pollutant.

APPENDIX D. Summary of Time–Activity Data for Study Subjects

Table D.1. Differences in Average Time (hr) Spent Traveling Between Visits

	Foot/Bicycle	Motorcycling	Car/Taxi	Bus	Train/Subway
Visit 1 (n = 125)					
Mean	1.06	0.00	0.05	0.78	0.08
SD	0.21	0.00	0.04	0.18	0.06
Visit 2 (n = 123)					
Mean	0.83	0.07	0.09	0.68	0.04
SD	0.18	0.06	0.06	0.17	0.04
Visit 3 (n = 125)					
Mean	0.87	0.00	0.14	0.80	0.23
SD	0.18	0.00	0.08	0.18	0.10
Visit 4 (n = 124)					
Mean	0.63	0.00	0.02	0.82	0.06
SD	0.16	0.00	0.03	0.18	0.05
Visit 5 (n = 123)					
Mean	0.69	0.00	0.07	0.61	0.08
SD	0.17	0.00	0.05	0.16	0.06
Visit 6 (n = 124)					
Mean	0.66	0.00	0.06	0.73	0.15
SD	0.16	0.01	0.05	0.17	0.08
P value	0.003	0.42	0.16	0.57	0.20
ICC (95% CI)[a]	0.31 (0.23 to 0.39)	0.00 (−0.04 to 0.05)	0.15 (0.09 to 0.22)	0.37 (0.30 to 0.46)	0.07 (0.02 to 0.13)

[a] ICC indicates intraclass correlation coefficient.

Table D.2. Differences in Average Time (hr) Spent Indoors and Outdoors Between Visits

	Inside Home	Inside Work	Inside Other Setting	Outside
Visit 1 (n = 125)				
Mean	13.42	6.26	1.20	0.69
SD	0.50	0.44	0.22	0.17
Visit 2 (n = 123)				
Mean	10.48	7.32	1.29	0.97
SD	0.50	0.46	0.23	0.20
Visit 3 (n = 125)				
Mean	11.43	7.46	1.36	0.45
SD	0.50	0.46	0.23	0.14
Visit 4 (n = 124)				
Mean	12.82	7.68	1.06	0.43
SD	0.50	0.47	0.21	0.13
Visit 5 (n = 123)				
Mean	11.99	8.09	1.41	0.43
SD	0.50	0.47	0.23	0.13
Visit 6 (n = 124)				
Mean	12.73	7.65	1.21	0.41
SD	0.50	0.47	0.22	0.13
P value	0.02	0.20	0.74	0.007
ICC (95% CI)[a]	0.20 (0.13 to 0.28)	0.23 (0.16 to 0.31)	0.20 (0.14 to 0.28)	0.21 (0.15 to 0.29)

[a] ICC indicates intraclass correlation coefficient.

Table D.3. Differences in Average Time (hr) Spent Engaged in Various Activities Between Visits

	Cooking	Sleeping	Exercising	At a Special Event	With Someone Smoking in the Same Room
Visit 1 (n = 125)					
Mean	0.05	8.10	0.53	0.06	0.03
SD	0.05	0.47	0.15	0.05	0.03
Visit 2 (n = 123)					
Mean	0.09	6.89	0.96	0.30	0.04
SD	0.06	0.45	0.20	0.11	0.04
Visit 3 (n = 125)					
Mean	0.07	8.16	0.28	0.40	0.05
SD	0.05	0.47	0.11	0.13	0.04
Visit 4 (n = 124)					
Mean	0.04	8.16	0.27	0.46	0.00
SD	0.04	0.47	0.10	0.14	0.00
Visit 5 (n = 123)					
Mean	0.07	8.22	0.39	0.48	0.01
SD	0.06	0.47	0.13	0.14	0.02
Visit 6 (n = 124)					
Mean	0.08	8.08	0.31	0.38	0.00
SD	0.06	0.47	0.11	0.13	0.00
P value	0.79	0.02	0.24	0.23	0.45
ICC (95% CI)[a]	0.16 (0.10 to 0.23)	0.24 (0.17 to 0.33)	0.31 (0.24 to 0.40)	0.35 (0.28 to 0.44)	−0.01 (−0.05 to 0.05)

[a] ICC indicates intraclass correlation coefficient.

APPENDIX E: Degrees of Freedom Selected for Meteorologic Parameters and *P* Values for Associations Between Temperature or RH and Biomarkers[a]

	Temperature		RH		Temperature Moving Average[b]		RH Moving Average[b]	
Biomarker	df	*P* Value	df	*P* Value	df (dy)	*P* Value	df (dy)	*P* Value
Autonomic Dysfunction								
HR (log)	1	0.070	1	0.21	—	NA	—	NA
HF (log)	1	0.12	1	0.60	1 (7)	0.77	—	NA
LF (log)	2	0.0004	1	0.043	1 (7)	0.22	1 (5)	0.0064
LF/HF (log)	2	0.17	1	0.054	1 (7)	0.14	1 (2)	0.022
rMSSD (log)	1	0.22	1	0.58	1 (7)	0.48	—	NA
SDNN (log)	1	0.31	1	0.76	1 (7)	0.28	—	NA
VLF (log)	1	0.31	1	0.89	1 (7)	0.28	—	NA
Total power (log)	1	0.63	1	0.11	1 (7)	0.17	1 (5)	0.027
DBP	3	0.091	3	0.78	3 (7)	0.66	3 (5)	0.017
SBP	3	0.19	2	0.010	3 (7)	0.041	3 (2)	0.027
Systemic Inflammation and Oxidative Stress								
CRP[b]	1	NA	1	NA	1 (7)	NA	2 (7)	NA
Fibrinogen	3	0.0002	1	0.17	1 (6)	0.022	—	NA
RBCs	1	0.0001	1	0.57	—	NA	1 (4)	0.0002
WBCs	1	0.0924	1	0.49	1 (7)	0.13	2 (7)	0.027
Lymphocytes	1	<0.0001	1	0.17	—	NA	—	NA
Neutrophils	1	0.28	1	0.42	1 (7)	0.24	—	NA
Urinary 8-OHdG (log)	1	0.37	1	0.11	1 (7)	0.72	3 (2)	0.19
Pulmonary Oxidative Stress and Inflammation								
FeNO (log)	2	0.0021	3	<0.0001	2 (7)	<0.0001	3 (7)	0.0001
EBC								
Nitrite (log)	2	0.0004	1	<0.0001	3 (7)	<0.0001	3 (3)	<0.0001
Nitrate (log)	2	<0.0001	1	<0.0001	3 (7)	<0.0001	3 (7)	<0.0001
Nitrite+nitrate (log)	1	<0.0001	3	<0.0001	3 (7)	<0.0001	3 (5)	<0.0001
pH	1	0.45	1	0.38	3 (6)	<0.0001	1 (3)	0.0010
8-Isoprostane[b]	1	NA	1	NA	2 (7)	NA	1 (2)	NA
Hemostasis								
sCD62P (log)	1	0.017	3	0.0005	2 (7)	0.85	2 (4)	0.0063
sCD40L (log)	1	0.43	1	0.57	1 (5)	0.011	1 (2)	0.24
Adrenaline	3	<0.0001	3	0.0097	3 (7)	0.72	3 (3)	<0.0001
vWF	3	0.0058	3	<0.0001	3 (7)	0.077	3 (6)	<0.0001

[a] *P* values were not available from the modeling software for the overall effects of temperature and RH.

[b] The number of days included in the moving average as well as the degrees of freedom chosen for the natural splines are listed in the df (dy) column. If the AIC was not minimized by adding a moving average to the model, then this term was not added, those cells contain a dash, and the corresponding *P* values are listed as not available (NA).

APPENDIX F. Biomarker Results from a Comparison of Two-Pollutant Models

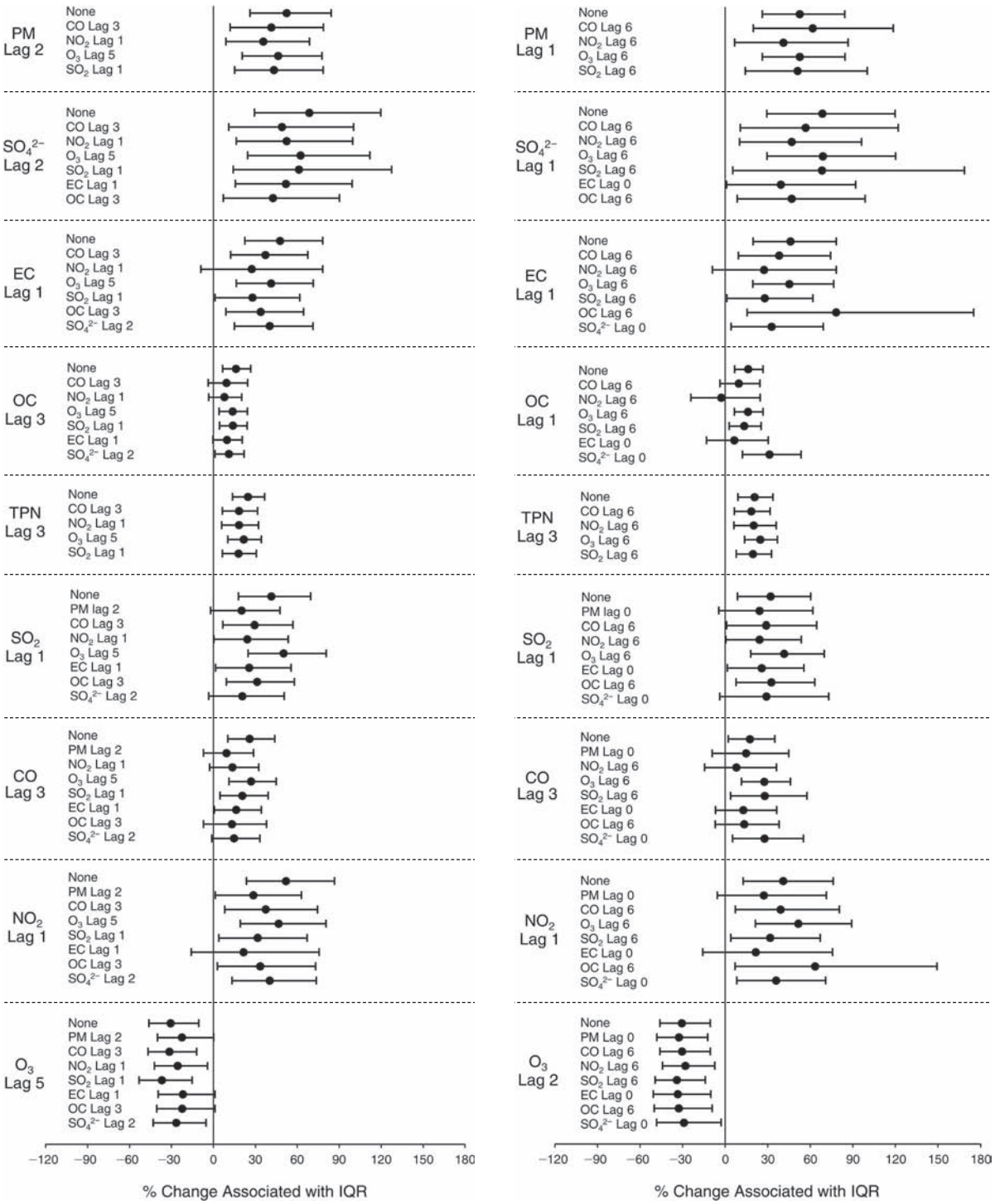


Figure F.1. Estimated means and 95% CIs for the percent change in urinary 8-OHdG controlling for temperature (df = 1), RH (df = 1), 7-day moving average for temperature (df = 1), 2-day moving average for RH (df = 3), sex, day of the week, and a second pollutant. Left: results of a two-pollutant model controlling for the second pollutant using the most significant lag day; **Right:** results of a two-pollutant model controlling for the second pollutant using the same lag day as the primary pollutant.

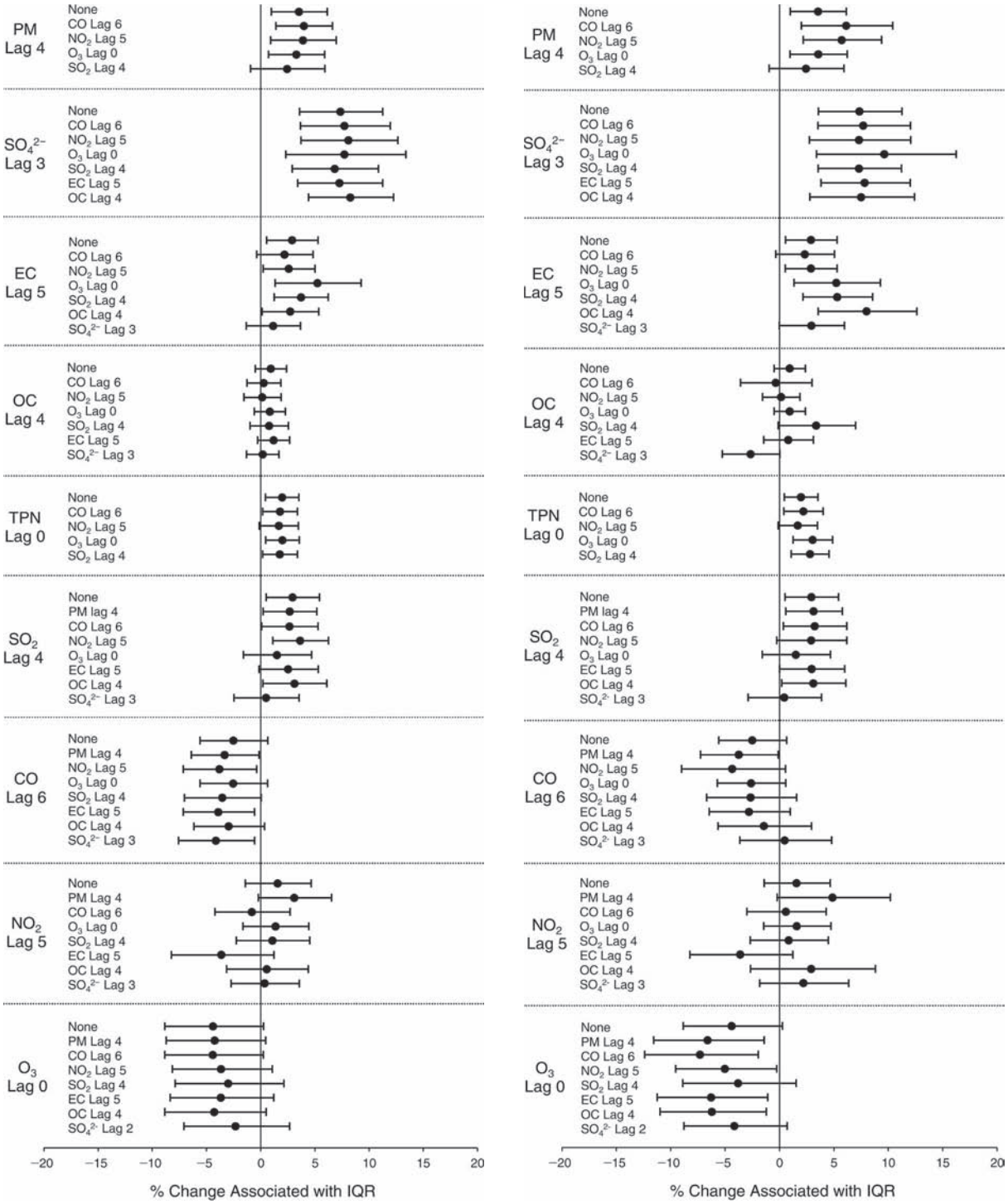


Figure F.2. Estimated means and 95% CIs for the percent change in sCD40L controlling for temperature (df = 1), RH (df = 1), 5-day moving average for temperature (df = 1), 2-day moving average for RH (df = 1), sex, day of the week, and a second pollutant. Left: results of a two-pollutant model controlling for the second pollutant using the most significant lag day; **Right:** results of a two-pollutant model controlling for the second pollutant using the same lag day as the primary pollutant.

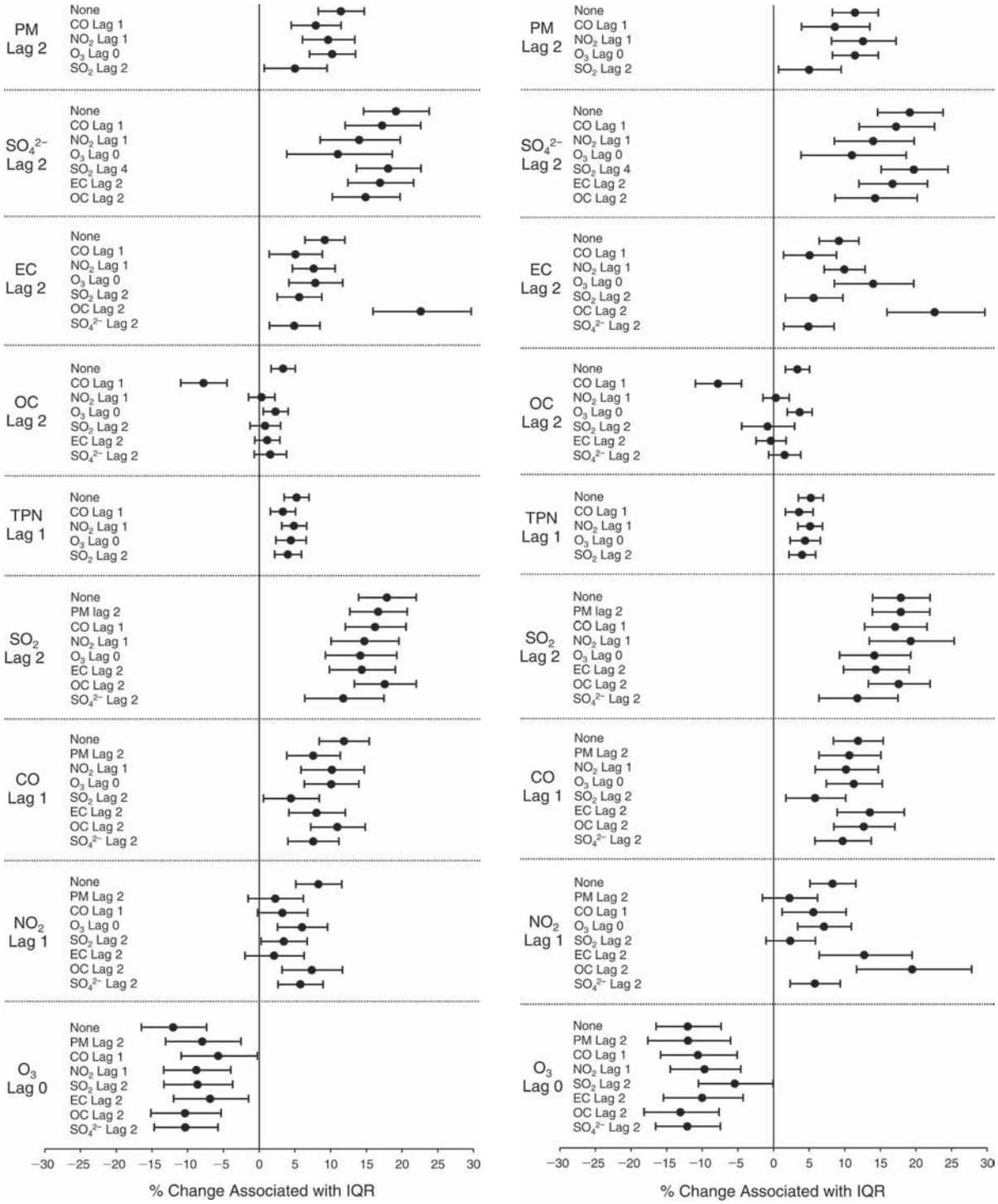


Figure F.3. Estimated means and 95% CIs for the percent change in sCD62P controlling for temperature (df = 1), RH (df = 3), 7-day moving average for temperature (df = 2), 4-day moving average for RH (df = 2), sex, day of the week, and a second pollutant. Left: results of a two-pollutant model controlling for the second pollutant using the most significant lag day; **Right:** results of a two-pollutant model controlling for the second pollutant using the same lag day as the primary pollutant.

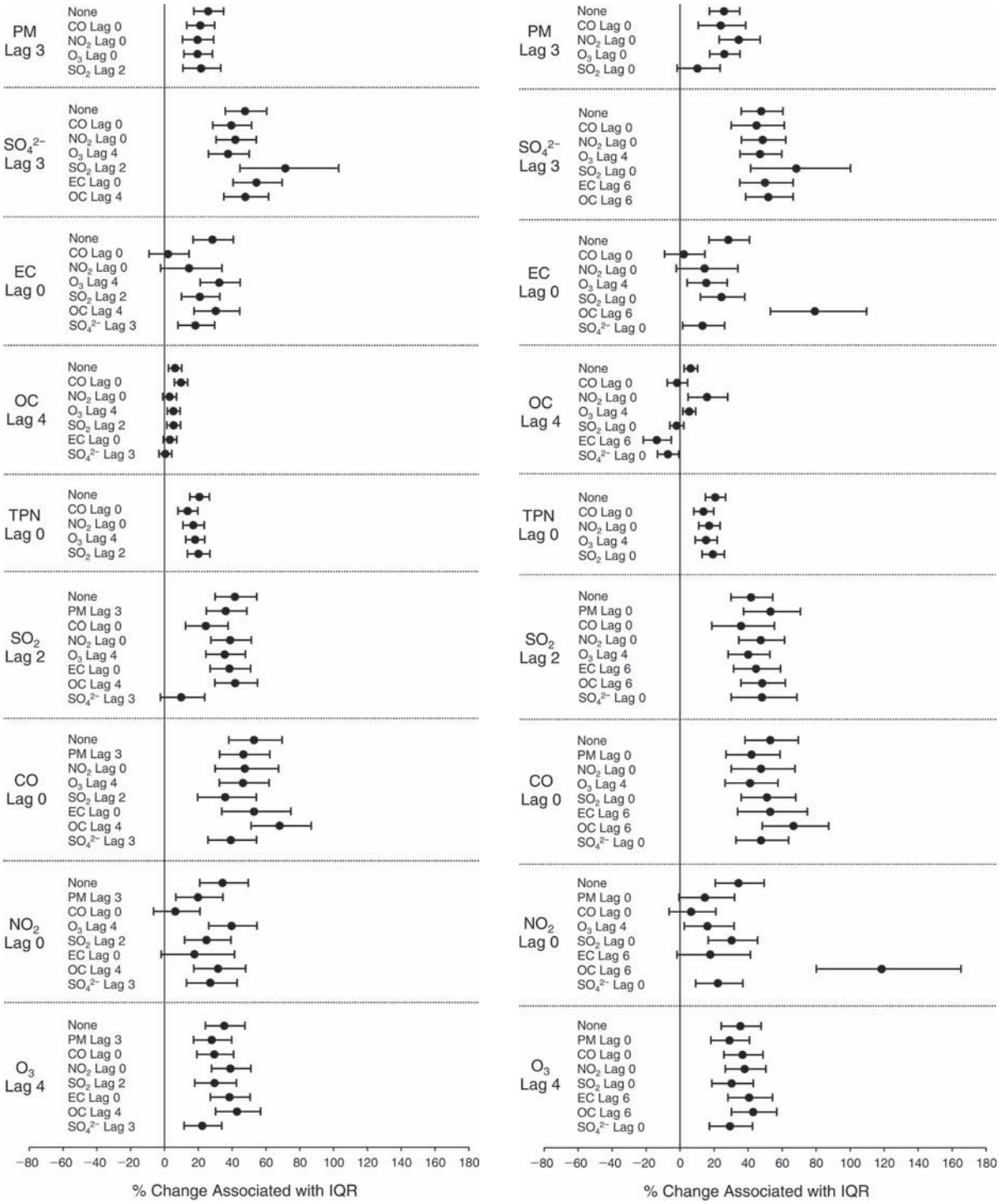


Figure F.4. Estimated means and 95% CIs for the percent change in FeNO controlling for temperature (df = 2), RH (df = 3), 7-day moving average for temperature (df = 2), 7-day moving average for RH (df = 3), sex, day of the week, and a second pollutant. Left: results of a two-pollutant model controlling for the second pollutant using the most significant lag day; **Right:** results of a two-pollutant model controlling for the second pollutant using the same lag day as the primary pollutant.

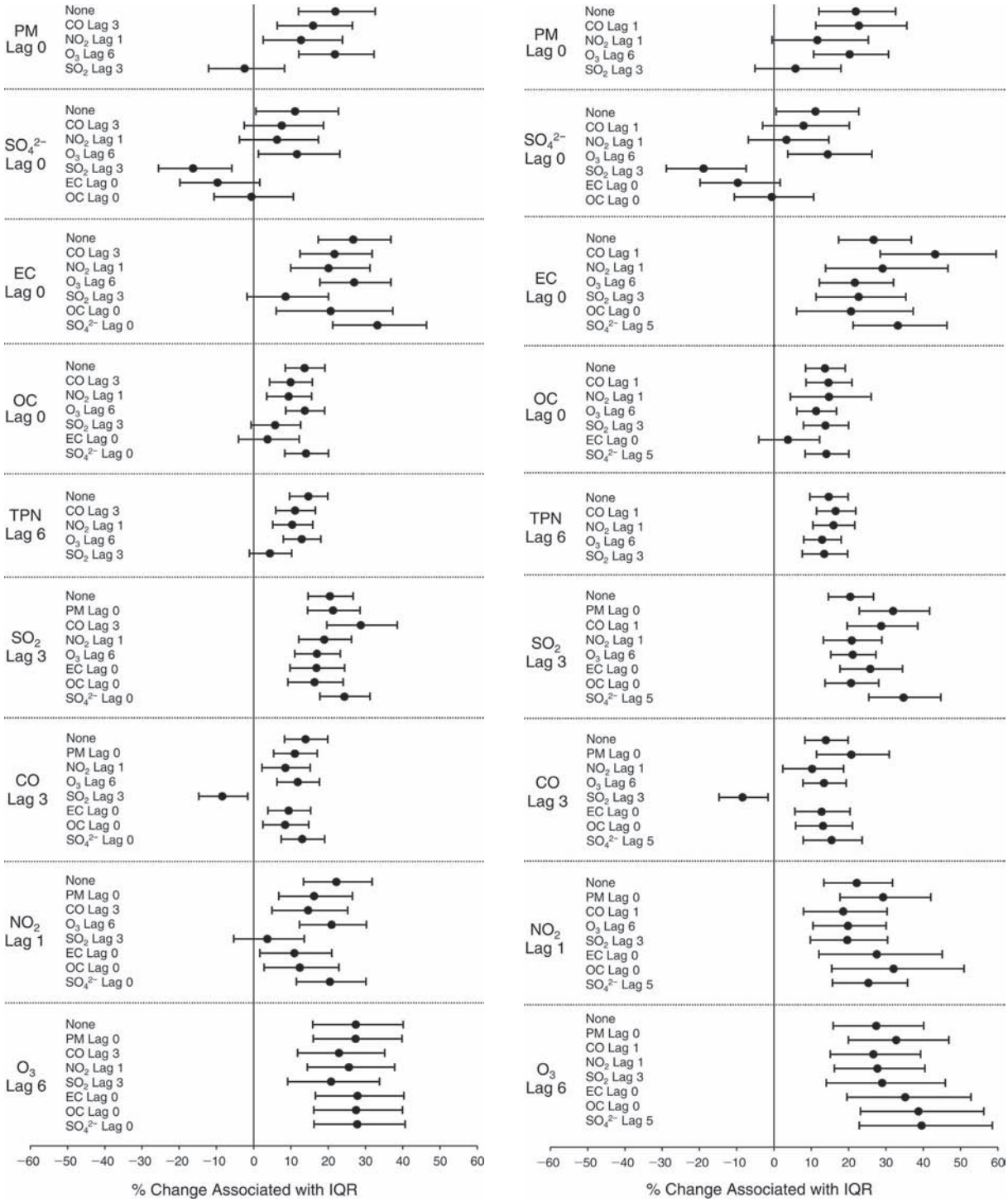


Figure F.5. Estimated means and 95% CIs for the percent change in EBC nitrite controlling for temperature (df = 2), RH (df = 1), 7-day moving average for temperature (df = 3), 3-day moving average for RH (df = 3), sex, day of the week, and a second pollutant. Left: results of a two-pollutant model controlling for the second pollutant using the most significant lag day; **Right:** results of a two-pollutant model controlling for the second pollutant using the same lag day as the primary pollutant.

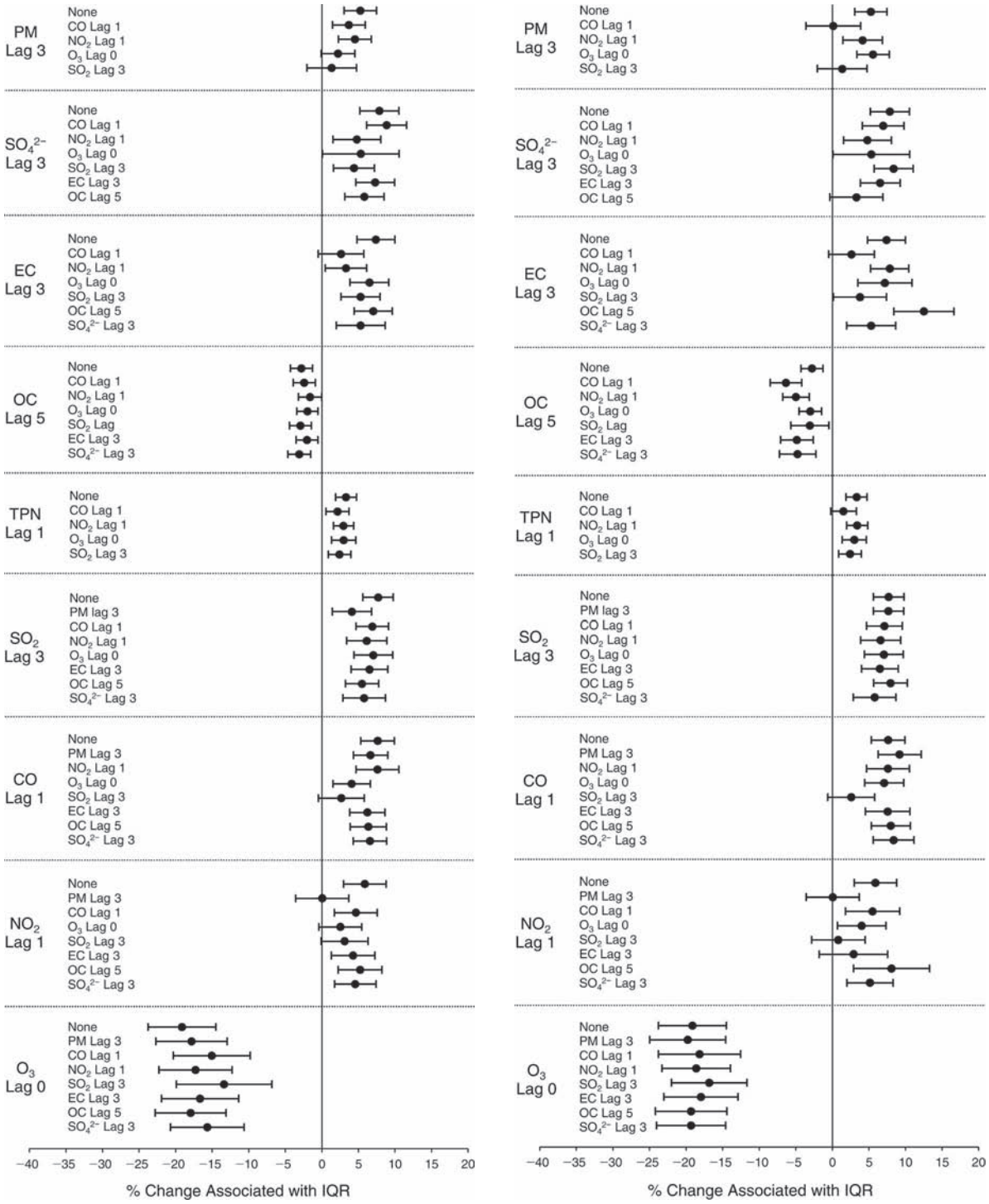


Figure F.6. Estimated means and 95% CIs for the percent change in vWF controlling for temperature (df = 3), RH (df = 3), 6-day moving average for temperature (df = 3), 6-day moving average for RH (df = 3), sex, day of the week, and a second pollutant. Left: results of a two-pollutant model controlling for the second pollutant using the most significant lag day; **Right:** results of a two-pollutant model controlling for the second pollutant using the same lag day as the primary pollutant.

APPENDIX G. Description of Statistical Models

This appendix describes the primary analyses for the biomarker outcomes. Modifications made in sensitivity analyses are described in the main report.

We used mixed model analyses to examine period (pre-, during-, and post-Olympics) effects as well as, separately, associations between biomarker levels, controlling for temperature, RH, sex, and day of the week. We used the following basic algebraic model for biomarkers measured as continuous outcomes, whether log-transformed or not:

$$Y_{ijk} = \alpha_i + h_T(TEMP_{ijk}) + h_{RH}(RH_{ijk}) + \beta_1 D_{1,ijk} + \beta_2 D_{2,ijk} + \beta_3 D_{3,ijk} + \beta_4 D_{4,ijk} + \beta_5 D_{5,ijk} + \beta_6 D_{6ijk} + \beta_7 S_i + \Sigma_{ijk}$$

where Y_{ijk} represents the jth observation (j = 1,2) in the kth period (k = 1,2,3) for the ith subject. We included sex (S_i = 1 if the ith subject was male and 0 if female) and day of the week (for example, $D_{1,ijk}$ = 1 if Sunday, 0 if not; $D_{2,ijk}$ = 1 if Monday, 0 if not, etc.) as categorical factors via indicator variables in all models. We used natural splines to adjust for the effects of temperature and RH for each biomarker (Hastie and Tibshirani, 1990). Specifically, the effects of same-day temperature and RH were included in all models. These are represented in the model given above as $h_T(TEMP_{ijk})$ and $h_{RH}(RH_{ijk})$ for temperature and RH, respectively, such that the degrees of freedom (see Appendix E) were specific for each meteorologic factor and biomarker. Then, if the AIC value was reduced, we added moving averages of temperature and/or RH. We chose only one moving average each for temperature and for RH, based on the model with the lowest AIC value, with the number of days extending back from the current day, for up to 7 days. We allowed up to 3 df to model the trend in association between pairs of variables (e.g., temperature and biomarker levels). The degrees of freedom were limited to 3 because in some cases (e.g., blood biomarkers) careful investigation of partial regression plots revealed that allowing a greater number of degrees of freedom resulted in bi- or tri-modal effects of temperature on predicted biomarker levels, which seemed to indicate overfitting. A summary of the degrees of freedom and the significance levels for each meteorologic factor is included in Appendix E. Once the base model was determined as described above, we added indicator variables for period and continuous pollutant levels in order to determine adjusted changes across periods or associations with continuous measures of the pollutants.

Some of the biomarkers had right-skewed distributions. Therefore, we took natural log transformations to stabilize variances and meet model assumptions. The results of the natural log transformations are found in Appendix E.

In order to account for correlation within subjects, the above model includes a random intercept for subject, inducing equicorrelation between all observations within subject. We compared three alternative correlation structures using AIC: (1) a single random effect for subject, which induced equicorrelation between all observations within subject; (2) multiple random effects for subject, as well as for session within subject, allowing observations within a session for a subject to be more highly correlated than between sessions within subject; and (3) use of a power function such that the correlation between observations was set to $\sigma^2\rho^{dj,j'}$, where $d_{j,j'}$ represented the number of days between two observations within subject, indexed by j and j'. The model that was consistently chosen as best across biomarkers was the simplest model in which a random effect for subject induced equicorrelation between all observations within subject. For details of estimation when the outcome was binary, see the software package documentation (R Development Core Team 2011) for the lmer routine (*http://cran.r-project.org/web/packages/lme4/lme4.pdf*).

All analyses were completed using R software programming (R Development Core Team 2011). Two sample R programs are given below: the first demonstrates the modeled analyses for a continuous outcome, and the second, for a binary outcome.

```
##########################################################
#  EBC Nitrite - PM
##########################################################
pm0<-matrix(0,7,9)
dat<-cbind(one$logEBCNitrite, one$pmlag1,
one$pmlag2, one$pmlag3, one$pmlag4, one$pmlag5,
one$pmlag6, one$pmlag7, one$TEMPHR24,
one$RHHR24, one$TEMPma7, one$RHma3,one$gender,
one$period,  one$wkday, one$id)
dimnames(dat)[[2]]<-c("logEBCNitrite", "pmlag1",
"pmlag2","pmlag3","pmlag4","pmlag5","pmlag6",
"pmlag7", "TEMPHR24", "RHHR24", "TEMPma7",
"RHma3", "gender","period",  "wkday", "id")
aa<-data.frame(dat)

for (i in 1:7) {
xx=aa[,(1+i)]
a1<-lme(logEBCNitrite  ~ xx + ns(TEMPHR24,2) +
ns(RHHR24,1)+ns(TEMPma7,3) + ns(RHma3,3) +
factor(gender) +factor(wkday), random=~1|id,
data=aa, na.action=na.omit)
ciu<- a1$coef$fixed[2]+1.96*sqrt(a1$var[2,2])
cil<- a1$coef$fixed[2]-1.96*sqrt(a1$var[2,2])
```

```
z<-(a1$coef$fixed[2]/sqrt(a1$var[2,2]))
p<-2*(1-pnorm(abs(z)))
pm0[i,]<-c(0,i-1, a1$coef$fixed[2],
sqrt(a1$var[2,2]), z, cil, ciu, p, AIC(a1))
}

pm<-cbind("logEBCNitrite", "pm",pm0)
dimnames(pm)[[2]]<-c("Biomarker", "Pollutants",
"Period", "Lag", "slope", "se", "z",
"ci.lower", "ci.upper", "p", "AIC")
pm


#=====================================
#  Categorical: EBC 8-ISO
#=====================================

one$u0<-1*(one$iso<1.56)
qt<-quantile(one$iso[one$iso>1.56],  probs =
seq(0, 1, 0.25), na.rm=T)
qt3<-quantile(one$iso,  probs = .75, na.rm=T)
# > qt3  75%  6.21
one$u1<-(one$iso>=qt3)*1
mean(one$u1, na.rm=T)  #[1] 0.2581522
```

```
#######################################################
#  EBC 8-ISO - pm
#######################################################
pm0<-matrix(0,7,9)

dat<-cbind(one$iso, one$u1, one$pmlag1,
one$pmlag2, one$pmlag3, one$pmlag4, one$pmlag5,
one$pmlag6, one$pmlag7, one$TEMPHR24,
one$RHHR24,one$TEMPma7,one$RHma2, one$gender,
one$period, one$wkday, one$id)
dimnames(dat)[[2]]<-c("iso", "u1", "pmlag1",
"pmlag2", "pmlag3", "pmlag4", "pmlag5",
"pmlag6", "pmlag7","TEMPHR24", "RHHR24",
"TEMPma7","RHma2","gender","period",  "wkday",
"id")
aa<-data.frame(dat)

for (i in 1:7) {
xx=aa[,(2+i)]
a1<-lmer(u1~xx+ ns(TEMPHR24,1) + ns(RHHR24,1)
+ns(TEMPma7,2) +ns(RHma2,1)+ factor(gender)
+ factor(wkday)+(1|id), data=aa,
na.action=na.omit, family="binomial")
ciu<- fixef(a1)[2] +1.96*sqrt(vcov(a1)[2,2])
cil<- fixef(a1)[2] -1.96*sqrt(vcov(a1)[2,2])

z<-fixef(a1)[2]/sqrt(vcov(a1)[2,2])
p<-2*(1-pnorm(abs(z)))
pm0[i,]<-c(0,i-1, fixef(a1)[2],
sqrt(vcov(a1)[2,2]), z, cil, ciu, p,
AIC(logLik(a1)))
}

pm<-cbind("8-ISO","pm",pm0)
dimnames(pm)[[2]]<-c("Biomarker", "Pollutants",
"Period", "Lag", "slope", "se", "z", "ci.lower",
"ci.upper", "p", "AIC")
pm
```

APPENDIX H: Spearman Correlation Coefficients Between Biomarker Pairs

	HR	HF	LF	LF/HF	rMSSD	SDNN	VLF	Total power	DBP	SBP	CRP	Fibrinogen	RBCs	WBCs	Lymphocytes	Neutrophils	Urinary 8-OHdG	FeNO	EBC nitrite	EBC nitrate	EBC nitrite+nitrate	EBC pH	EBC 8-isoprostane	sCD62P	sCD40L	Platelet aggregation
HF	−0.527	1																								
LF	−0.296	0.504	1																							
LF/HF	0.349	−0.635	0.285	1																						
rMSSD	−0.571	0.764	0.451	−0.420	1																					
SDNN	−0.514	0.652	0.623	−0.170	0.873	1																				
VLF	−0.205	0.182	0.396	0.136	0.241	0.462	1																			
Total power	−0.465	0.768	0.764	−0.176	0.662	0.783	0.652	1																		
DBP	0.184	−0.214	−0.001	0.243	−0.211	−0.121	0.015	−0.095	1																	
SBP	0.047	−0.268	0.099	0.385	−0.173	−0.003	0.145	−0.026	0.619	1																
CRP	0.065	−0.107	−0.015	0.110	−0.091	−0.066	0.034	−0.052	0.230	0.237	1															
Fibrinogen	0.150	0.001	−0.121	−0.119	−0.082	−0.125	−0.068	−0.071	0.042	−0.087	0.336	1														
RBC	−0.015	−0.218	0.117	0.357	−0.045	0.111	0.220	0.018	0.284	0.510	0.183	−0.195	1													
WBC	0.086	0.061	0.080	−0.002	−0.011	−0.012	−0.004	0.053	0.101	0.090	0.193	0.125	0.053	1												
Lymphocytes	−0.034	0.032	0.054	−0.011	−0.009	−0.025	−0.028	0.015	0.076	0.007	0.087	−0.037	0.082	0.519	1											
Neutrophils	0.129	0.059	0.067	−0.006	−0.011	−0.005	−0.010	0.048	0.111	0.108	0.173	0.177	0.022	0.921	0.202	1										
Urine 8-OHdG	0.041	−0.043	−0.017	0.032	−0.015	−0.045	0.071	−0.018	−0.012	−0.030	−0.023	0.043	−0.100	−0.007	0.027	−0.038	1									
FeNO	−0.049	−0.017	0.020	0.023	0.008	0.059	0.077	0.042	0.048	0.153	−0.013	0.044	0.095	0.007	−0.141	0.058	−0.015	1								
EBC nitrite	−0.032	0.045	0.117	0.053	0.071	0.077	0.085	0.095	−0.019	0.008	0.060	−0.080	0.041	0.013	−0.010	0.005	0.003	0.087	1							
EBC nitrate	−0.027	0.014	−0.008	−0.031	−0.003	0.007	0.029	0.027	−0.001	0.044	0.029	0.105	0.006	−0.018	0.026	−0.030	0.024	0.077	0.004	1						
EBC nitrite+nitrate	−0.062	0.074	0.067	−0.023	0.057	0.068	0.096	0.101	−0.015	0.041	0.073	0.073	0.015	−0.005	−0.013	−0.010	0.028	0.142	0.666	0.648	1					
EBC pH	−0.044	−0.006	0.013	0.013	0.005	0.047	0.060	0.050	0.020	0.026	−0.057	0.024	0.090	−0.045	−0.057	−0.021	−0.069	0.005	0.023	−0.003	0.079	1				
EBC 8-isoprostane	0.004	0.034	0.009	−0.025	−0.012	−0.007	−0.001	0.036	0.026	−0.011	0.005	0.063	−0.054	−0.031	−0.026	−0.043	0.023	0.115	0.040	0.081	0.118	0.055	1			
sCD62P	0.010	0.015	0.033	0.022	0.014	0.015	0.029	0.028	−0.011	−0.021	−0.100	−0.047	0.019	−0.008	0.022	−0.030	0.117	0.184	0.125	0.088	0.102	−0.068	0.100	1		
sCD40L	0.000	0.011	0.018	0.010	0.021	0.027	−0.024	0.021	0.048	0.031	0.050	0.033	−0.014	−0.012	−0.054	0.001	0.055	0.094	−0.047	0.113	0.047	−0.029	0.051	0.072	1	
Platelet aggregation	0.026	0.050	−0.058	−0.100	0.044	0.004	−0.087	−0.042	−0.125	−0.264	0.015	0.095	−0.199	−0.042	−0.037	−0.045	−0.033	−0.117	−0.072	−0.009	−0.035	0.021	0.064	−0.080	−0.007	1
vWF	0.101	−0.137	−0.049	0.122	−0.123	−0.104	0.034	−0.079	−0.023	−0.075	0.173	0.084	0.069	−0.049	−0.062	−0.070	0.066	0.043	0.145	−0.033	0.089	−0.008	0.010	0.073	−0.047	0.062

APPENDIX I: Intraclass Correlations for Biomarkers by Period[a]

Biomarker	Pre-Olympics Mean (95% CI)	During-Olympics Mean (95% CI)	Post-Olympics Mean (95% CI)
Autonomic Dysfunction			
HR (bpm)[b]	0.51 (0.36 to 0.63)	0.62 (0.49 to 0.72)	0.65 (0.54 to 0.74)
HF (ms^2)[b]	0.61 (0.48 to 0.71)	0.69 (0.57 to 0.78)	0.50 (0.35 to 0.62)
LF (ms^2)[b]	0.50 (0.35 to 0.63)	0.39 (0.21 to 0.54)	0.45 (0.30 to 0.58)
LF/HF[b]	0.64 (0.52 to 0.74)	0.54 (0.39 to 0.66)	0.53 (0.39 to 0.65)
rMSSD (ms)[b]	0.57 (0.43 to 0.68)	0.31 (0.13 to 0.47)	0.61 (0.49 to 0.71)
SDNN (ms)[b]	0.58 (0.45 to 0.69)	0.35 (0.17 to 0.51)	0.58 (0.45 to 0.69)
VLF (ms^2)[b]	0.13 (−0.06 to 0.31)	0.29 (0.10 to 0.46)	0.20 (0.02 to 0.36)
Total power (ms^2)[b]	0.47 (0.31 to 0.60)	0.54 (0.39 to 0.66)	0.38 (0.22 to 0.52)
DBP (mmHg)	0.65 (0.54 to 0.74)	0.72 (0.62 to 0.80)	0.63 (0.51 to 0.73)
SBP (mmHg)	0.79 (0.71 to 0.85)	0.82 (0.75 to 0.87)	0.71 (0.61 to 0.79)
Systemic Inflammation and Oxidative Stress			
CRP (% ≥ 0.3 mg/L)	NA	NA	NA
Fibrinogen (g/L)	0.49 (0.34 to 0.61)	0.65 (0.53 to 0.74)	0.54 (0.40 to 0.65)
RBCs ($\times 10^{12}$/L)	0.87 (0.82 to 0.91)	0.93 (0.90 to 0.95)	0.93 (0.90 to 0.95)
WBCs ($\times 10^9$/L)	0.64 (0.52 to 0.73)	0.69 (0.58 to 0.77)	0.54 (0.40 to 0.65)
Lymphocytes ($\times 10^9$/L)	0.71 (0.61 to 0.79)	0.80 (0.73 to 0.86)	0.74 (0.65 to 0.81)
Neutrophils ($\times 10^9$/L)	0.57 (0.44 to 0.68)	0.56 (0.42 to 0.67)	0.42 (0.26 to 0.56)
Urinary 8-OHdG (mg/mol creatinine)[b]	0.02 (−0.16 to 0.20)	0.02 (−0.16 to 0.20)	0.39 (0.22 to 0.54)
Pulmonary Oxidative Stress and Inflammation			
FeNO (ppb)[b]	0.40 (0.24 to 0.54)	0.34 (0.17 to 0.49)	0.24 (0.07 to 0.40)
EBC			
Nitrite (μM)[b]	0.18 (0.01 to 0.34)	−0.07 (−0.25 to 0.11)	0.07 (−0.16 to 0.29)
Nitrate (μM)[b]	0.04 (−0.13 to 0.21)	−0.17 (−0.34 to 0.01)	−0.07 (−0.29 to 0.16)
Nitrite+nitrate (μM)[b]	0.14 (−0.04 to 0.31)	−0.13 (−0.30 to 0.05)	−0.04 (−0.26 to 0.19)
pH	−0.04 (−0.21 to 0.14)	0.20 (0.02 to 0.37)	0.04 (−0.14 to 0.21)
8-Isoprostane (% ≥ 1.56 pg/ml)	NA	NA	NA
Hemostasis			
sCD62P (ng/mL)[b]	0.07 (−0.11 to 0.24)	0.40 (0.24 to 0.54)	0.38 (0.22 to 0.52)
sCD40L (ng/mL)[b]	0.02 (−0.16 to 0.19)	0.22 (0.04 to 0.38)	−0.19 (−0.36 to −0.01)
Platelet aggregation (%)	0.55 (0.42 to 0.66)	0.50 (0.35 to 0.62)	0.35 (0.18 to 0.49)
vWF (%)	0.79 (0.71 to 0.85)	0.79 (0.71 to 0.85)	0.73 (0.64 to 0.80)

[a] NA indicates not available.
[b] Log transformation applied.

APPENDIX J. Estimated Means and 95% Confidence Intervals for Percent Change in Biomarker Levels from Period to Period, Controlling for Temperature, RH, and Their Moving Averages Having Statistically Significant Effects on Biomarkers

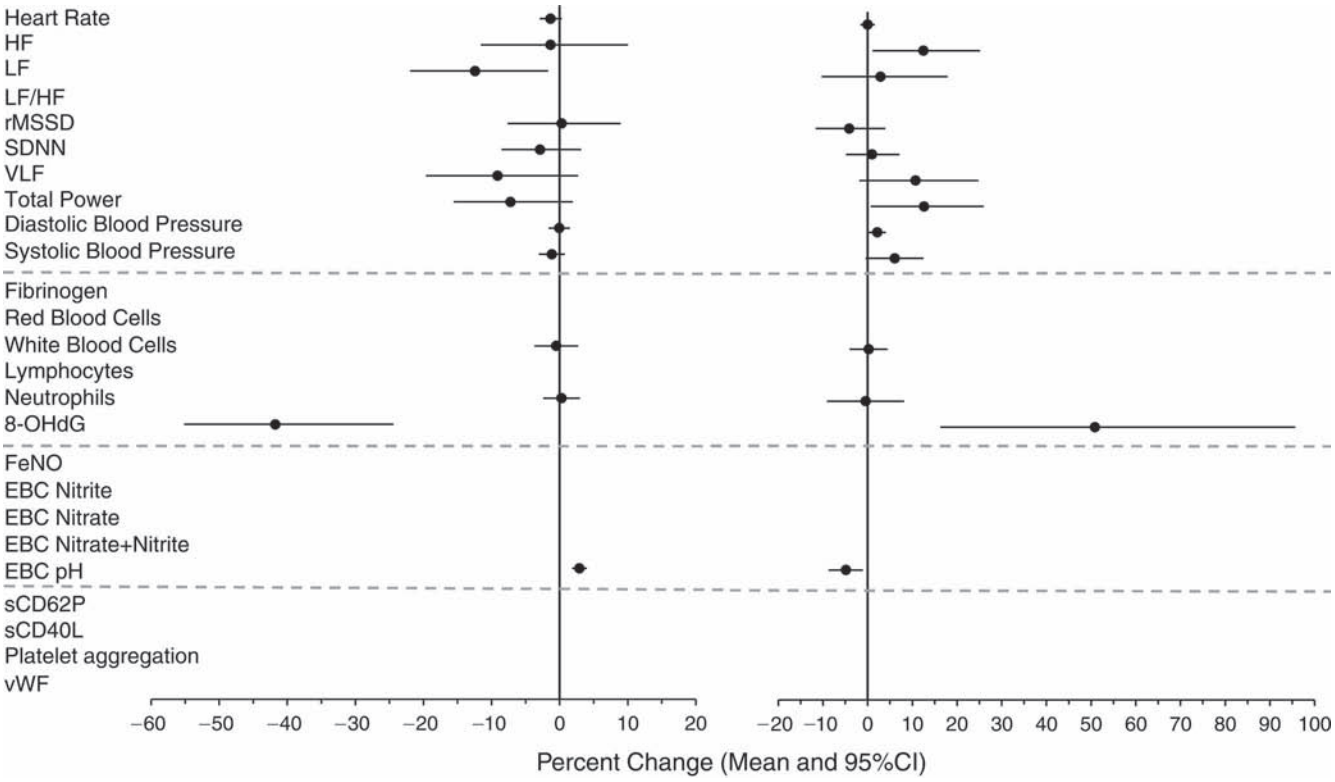


Figure J.1. Estimated means and 95% CIs for the percent change in biomarker levels from (left) the pre-Olympics to the during-Olympics period and (right) the during-Olympics to the post-Olympics period, controlling for temperature, RH, and their moving averages having statistically significant effects on biomarkers.

APPENDIX K. Percent Change in Biomarker Levels Associated with One IQR Increase in Pollutant Concentration With and Without Controlling for Temperature and RH

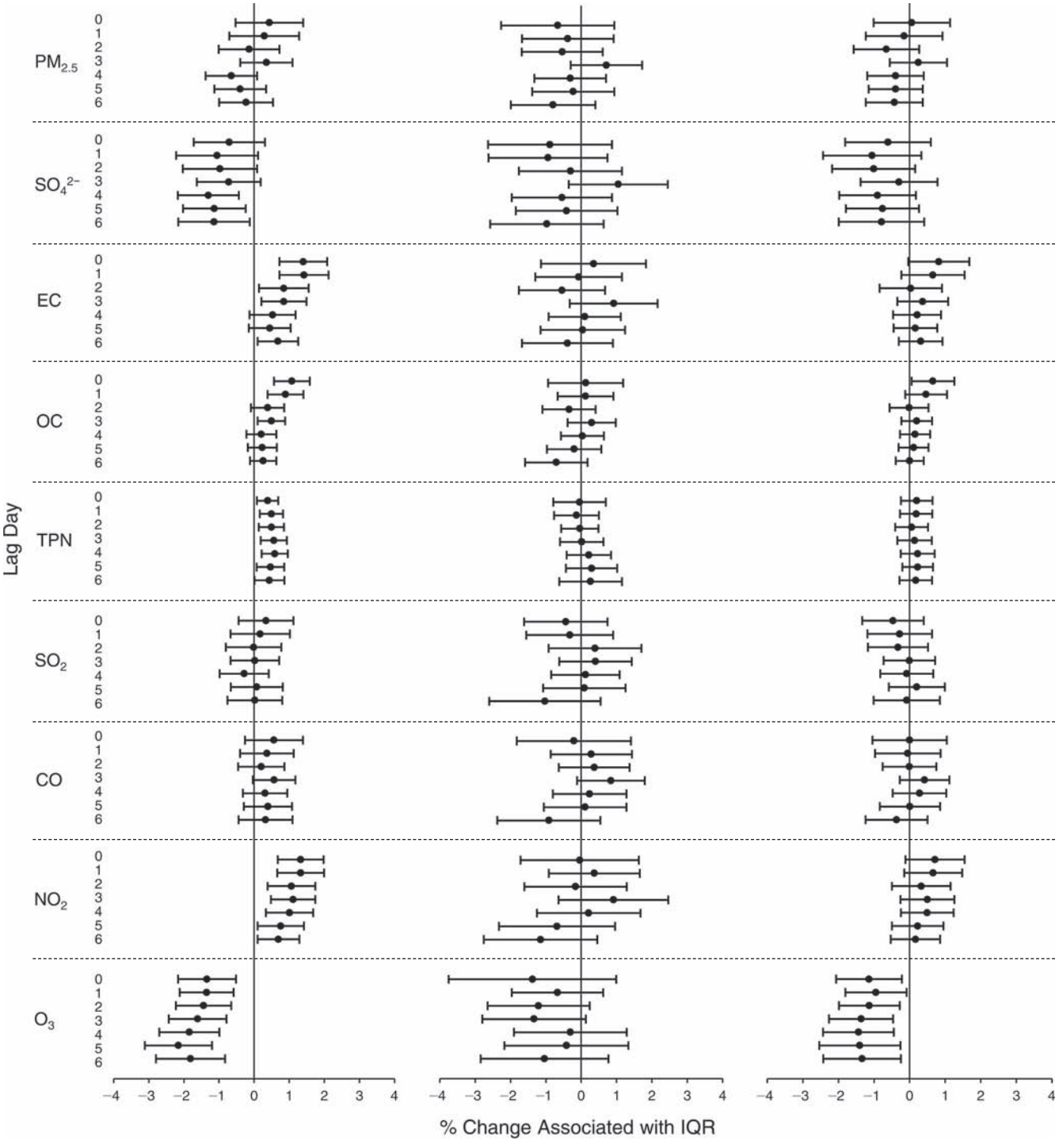


Figure K.1. Estimated means and 95% CIs for the percent change in DBP associated with one IQR increase in pollutant concentration. Left: results without any adjustment for temperature or RH; **Middle:** results with full adjustments; **Right:** results with adjustments for temperature and RH having statistically significant effects on biomarkers.

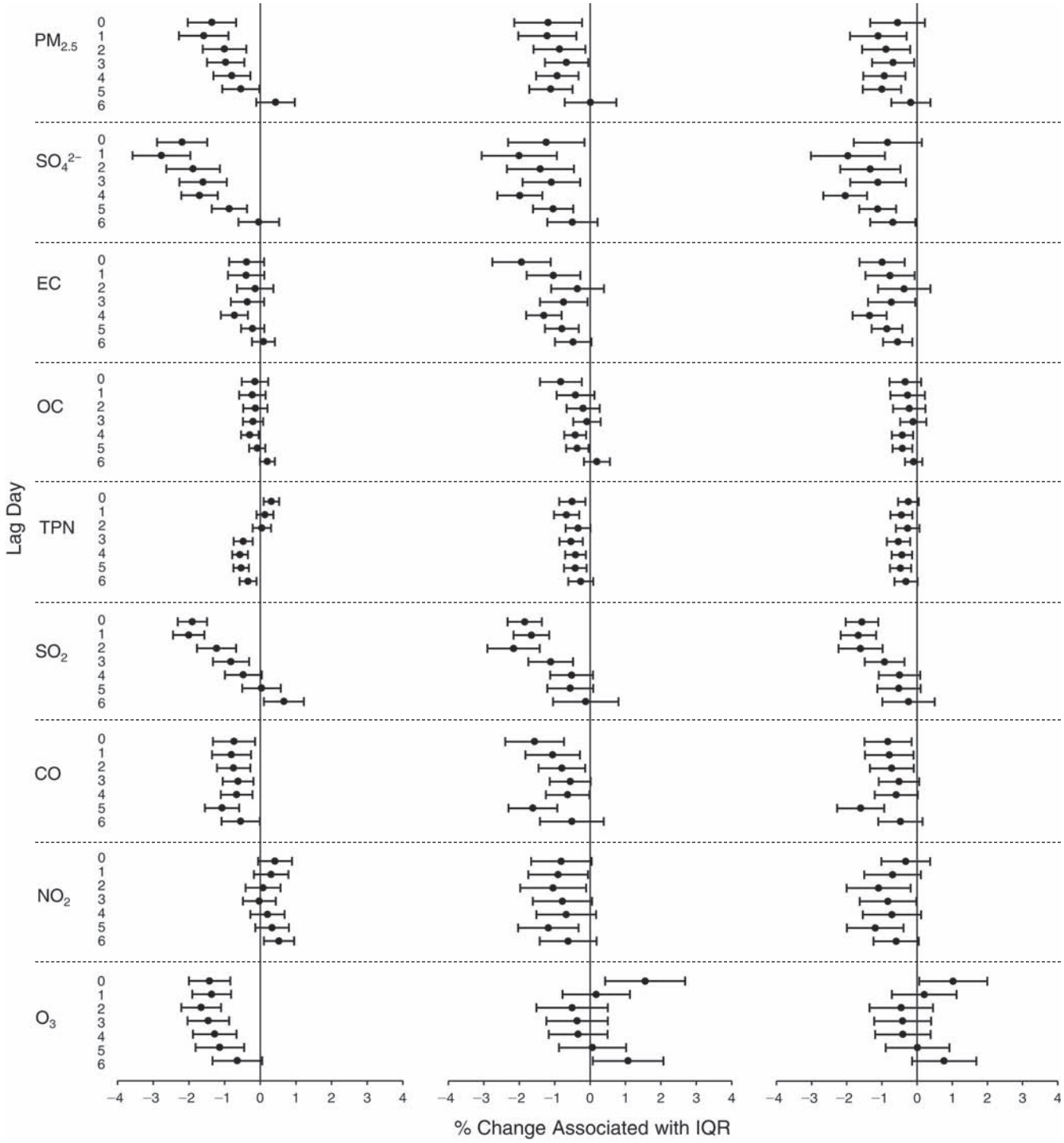


Figure K.2. Estimated means and 95% CIs for the percent change in EBC pH associated with one IQR increase in pollutant concentration. Left: results without any adjustment for temperature or RH; **Middle:** results with full adjustments; **Right:** results with adjustments for temperature and RH having statistically significant effects on biomarkers.

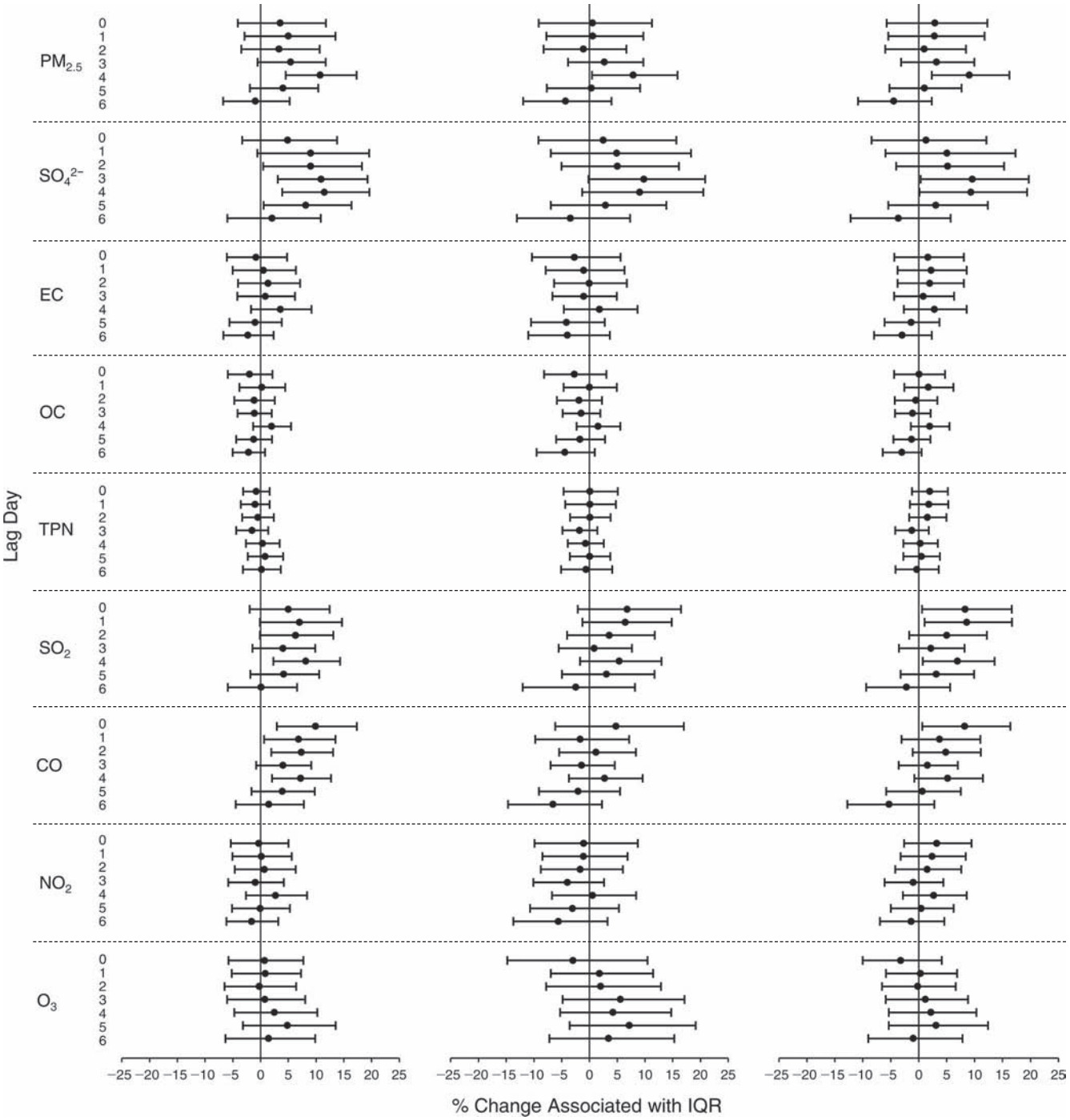


Figure K.3. Estimated means and 95% CIs for the percent change in LF (HRV) associated with one IQR increase in pollutant concentration. Left: results without any adjustment for temperature or RH; **Middle:** results with full adjustments; **Right:** results with adjustments for temperature and RH having statistically significant effects on biomarkers.

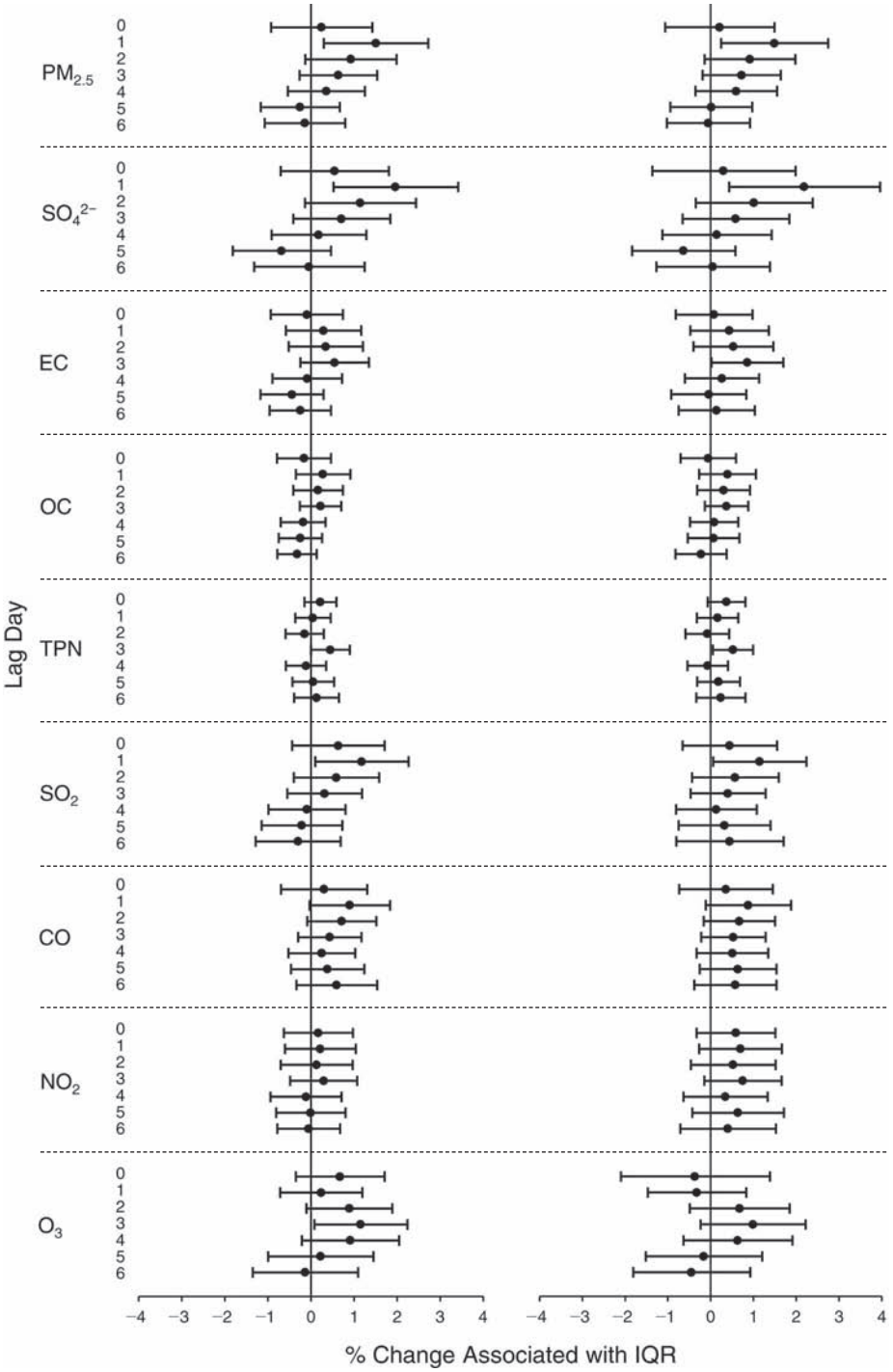


Figure K.4. Estimated means and 95% CIs for the percent change in HR associated with one IQR increase in pollutant concentration. Left: results without any adjustment for temperature or RH; **Right:** results with full adjustments.

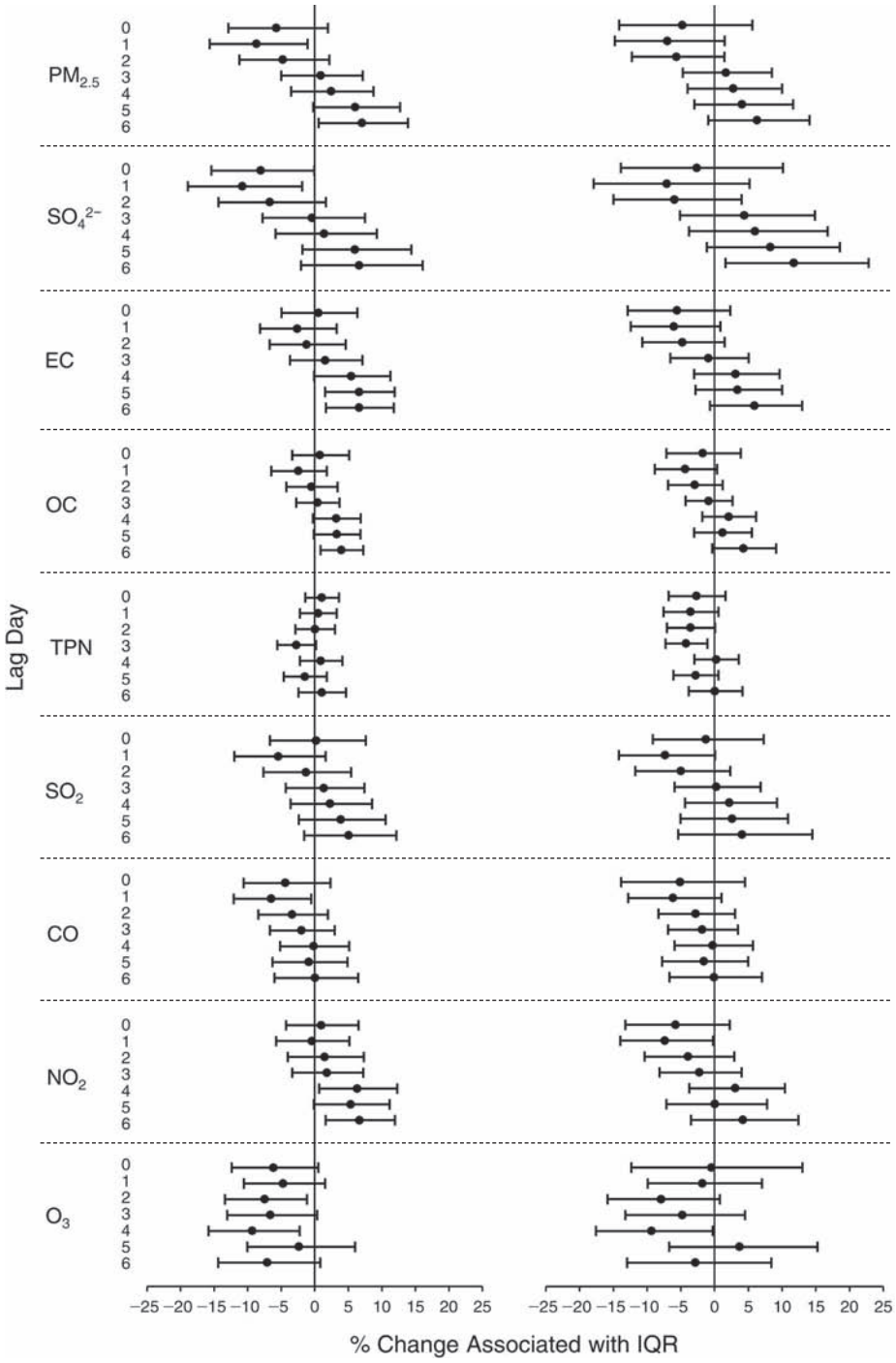


Figure K.5. Estimated means and 95% CIs for the percent change in HF (HRV) associated with one IQR increase in pollutant concentration. Left: results without any adjustment for temperature or RH; **Right:** results with full adjustments.

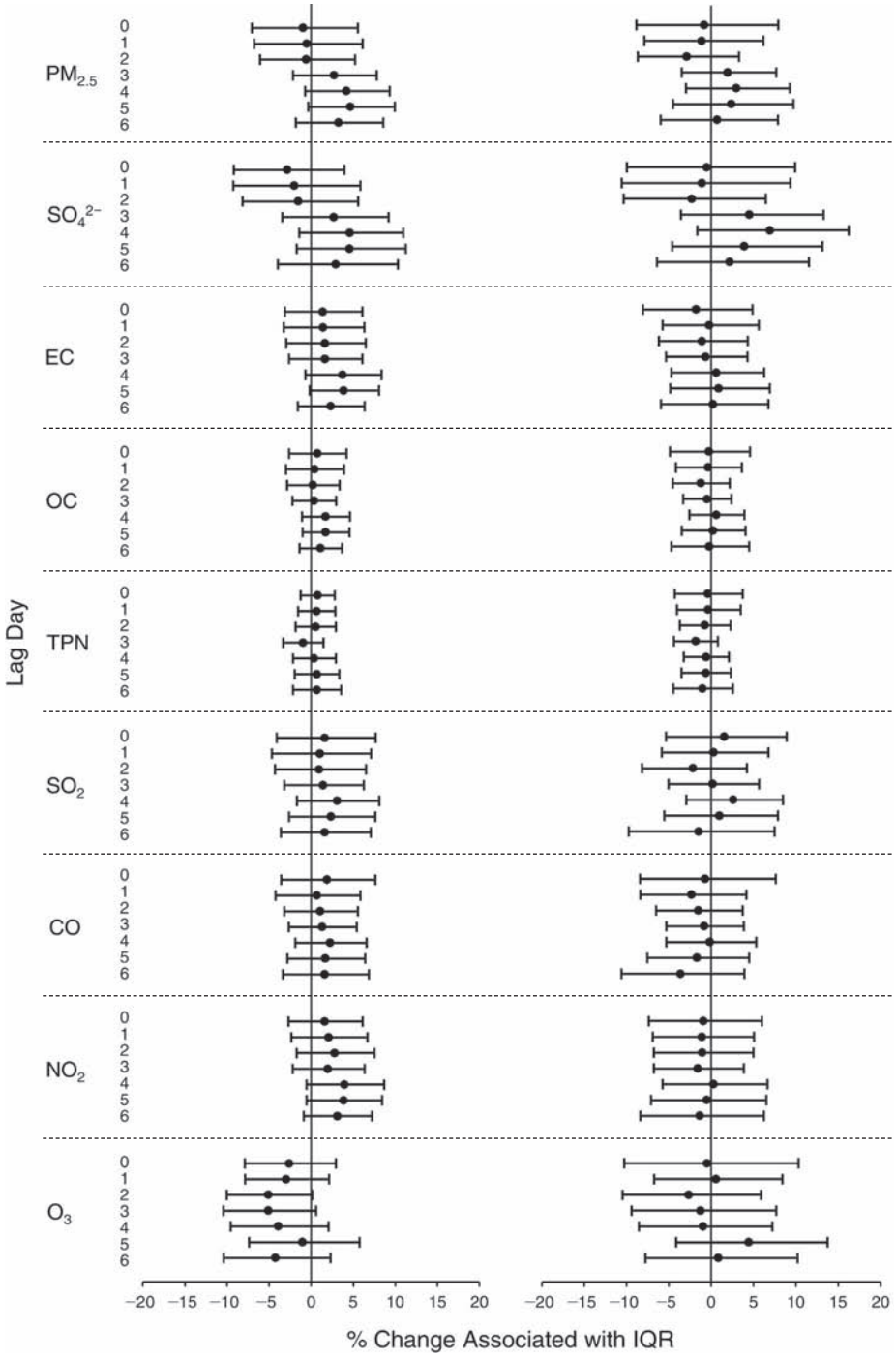


Figure K.6. Estimated means and 95% CIs for the percent change in total power (HRV) associated with one IQR increase in pollutant concentration. Left: results without any adjustment for temperature or RH; **Right:** results with full adjustments.

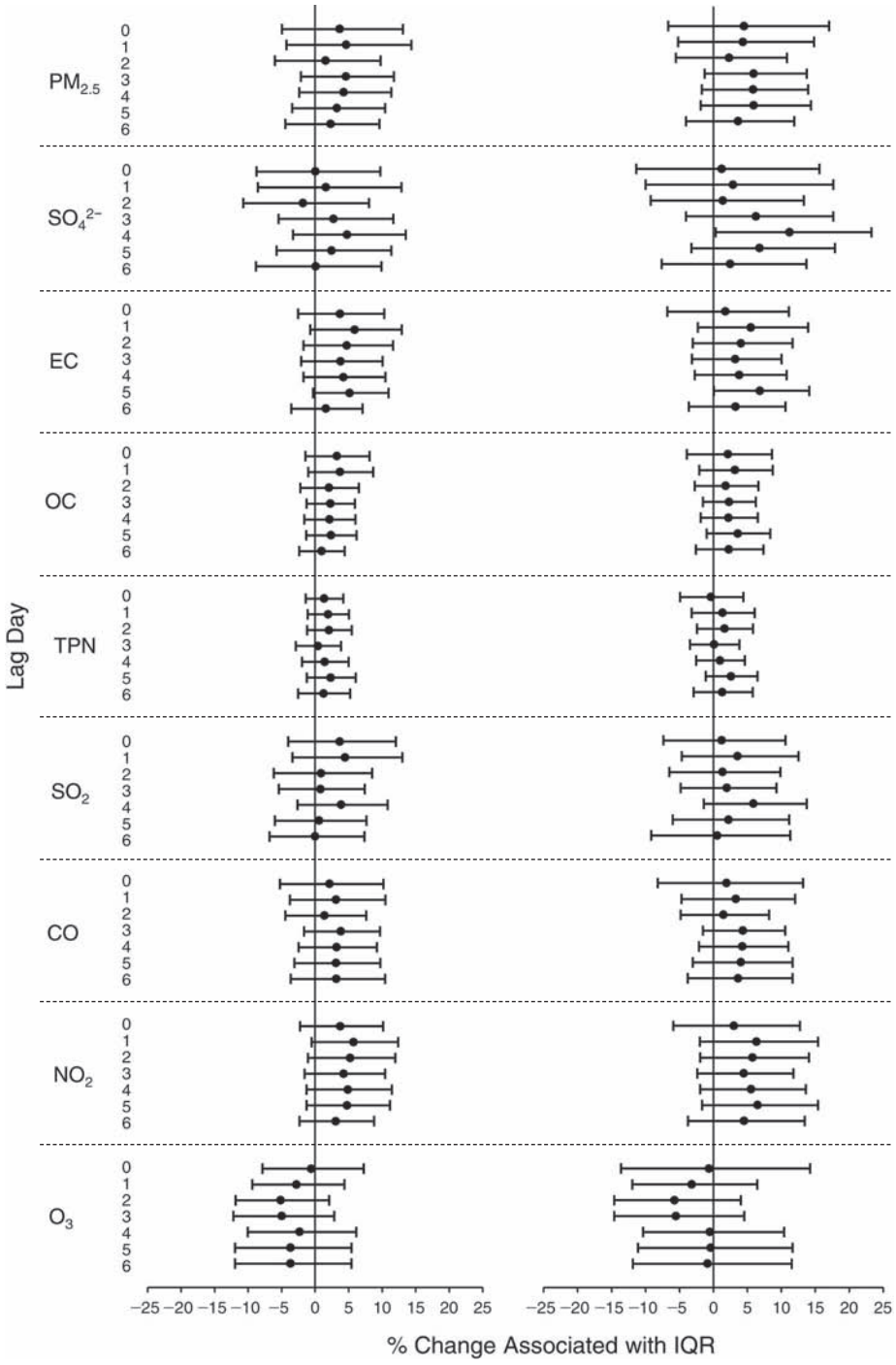


Figure K.7. Estimated means and 95% CIs for the percent change in VLF (HRV) associated with one IQR increase in pollutant concentration. Left: results without any adjustment for temperature or RH; **Right:** results with full adjustments.

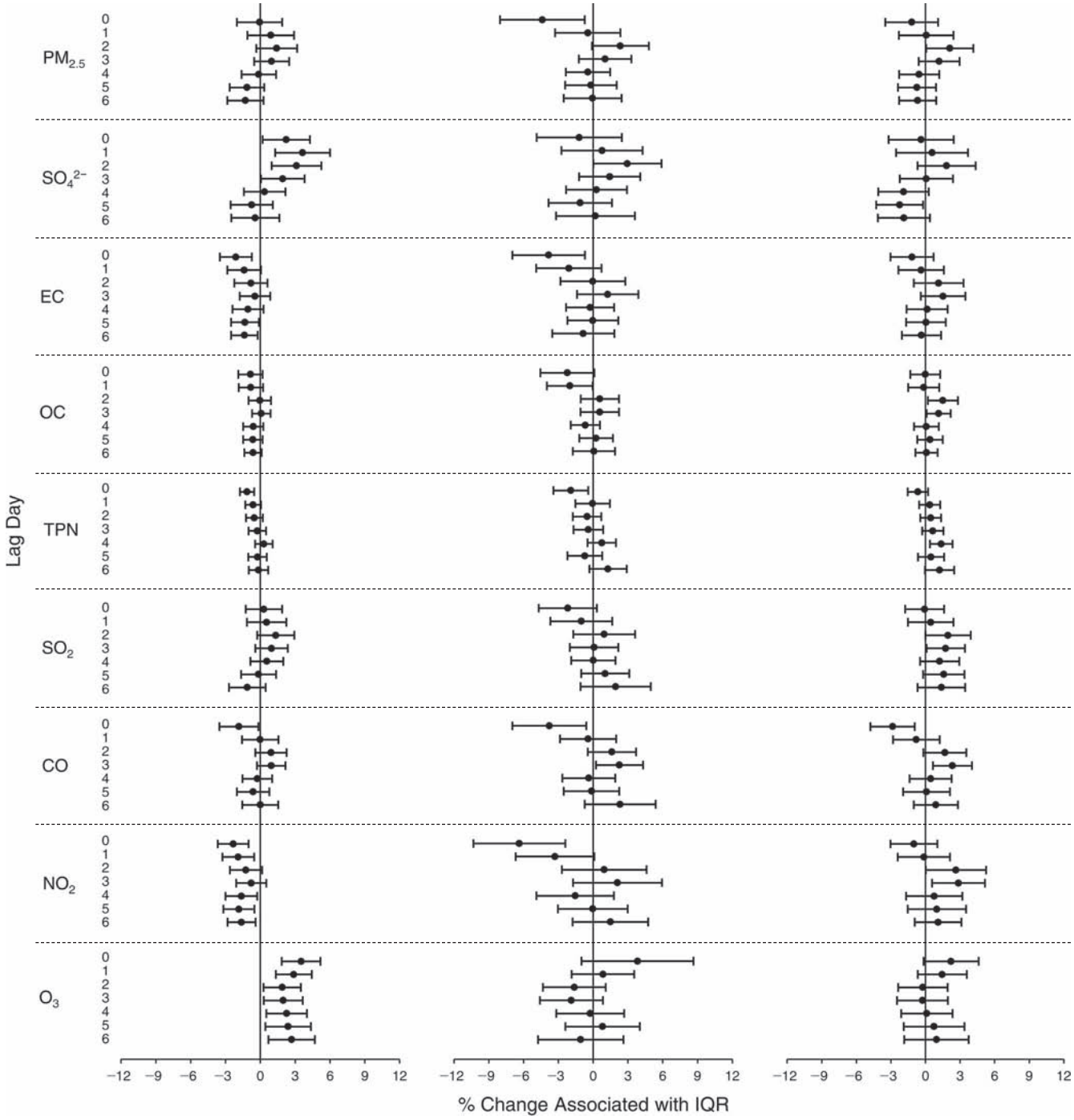


Figure K.8. Estimated means and 95% CIs for the percent change in lymphocyte count associated with one IQR increase in pollutant concentration. Left: results without any adjustment for temperature or RH; **Middle:** results with full adjustments; **Right:** results with adjustments for temperature and RH having statistically significant effects on biomarkers.

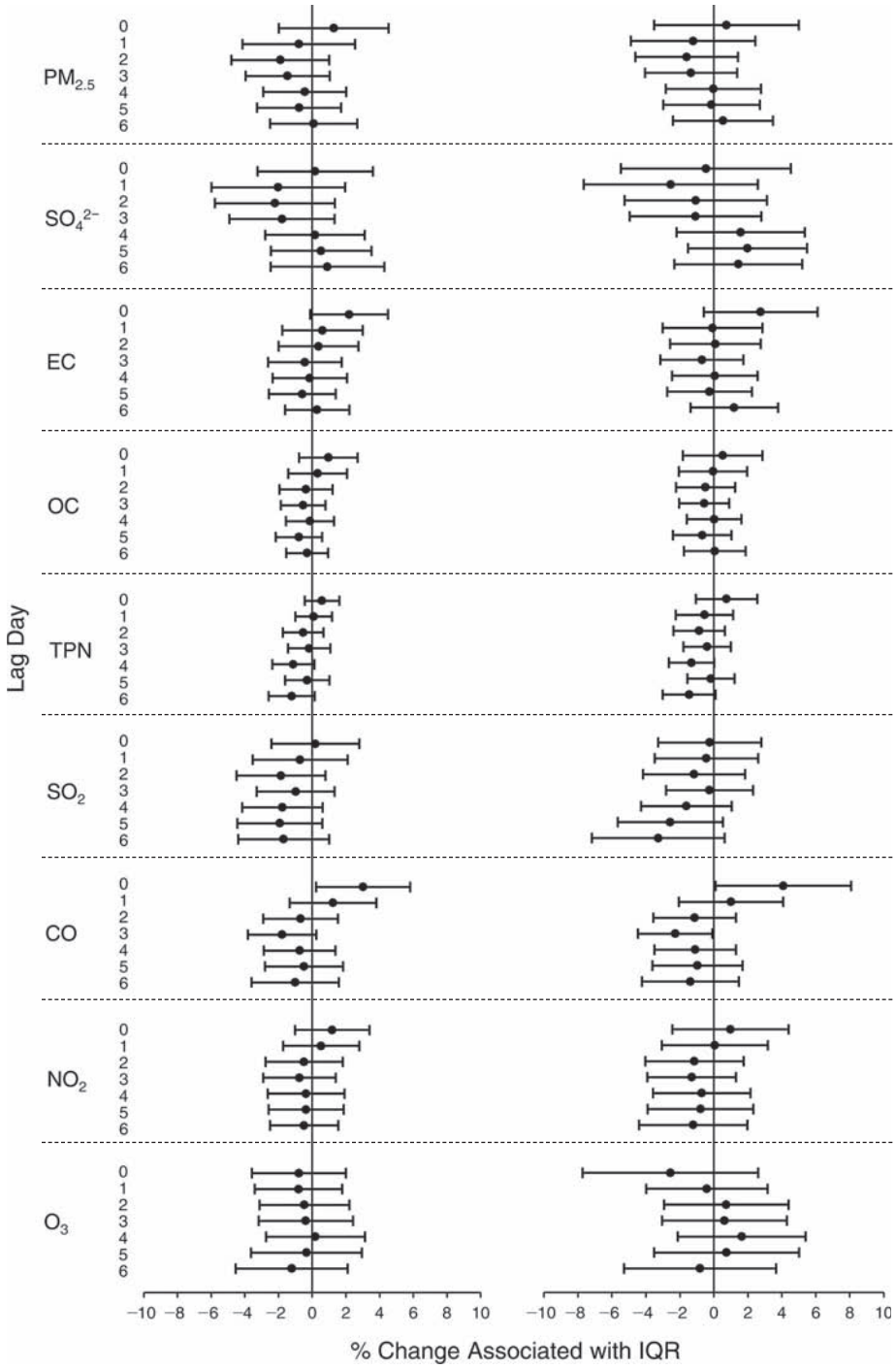


Figure K.9. Estimated means and 95% CIs for the percent change in neutrophil count associated with one IQR increase in pollutant concentration. Left: results without any adjustment for temperature or RH; **Right:** results with full adjustments.

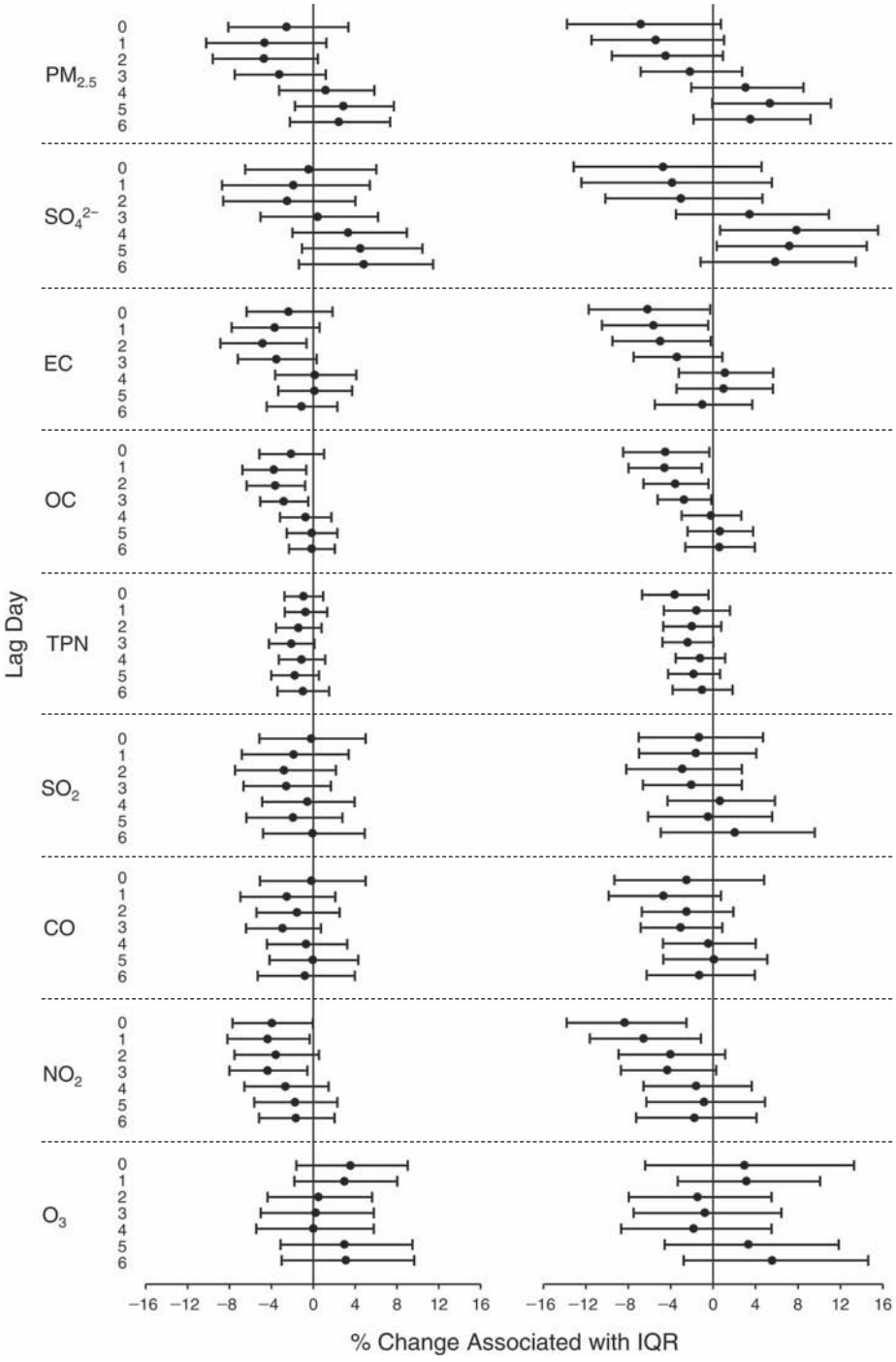


Figure K.10. Estimated means and 95% CIs for the percent change in rMSSD (HRV) associated with one IQR increase in pollutant concentration. Left: results without any adjustment for temperature or RH; **Right:** results with full adjustments.

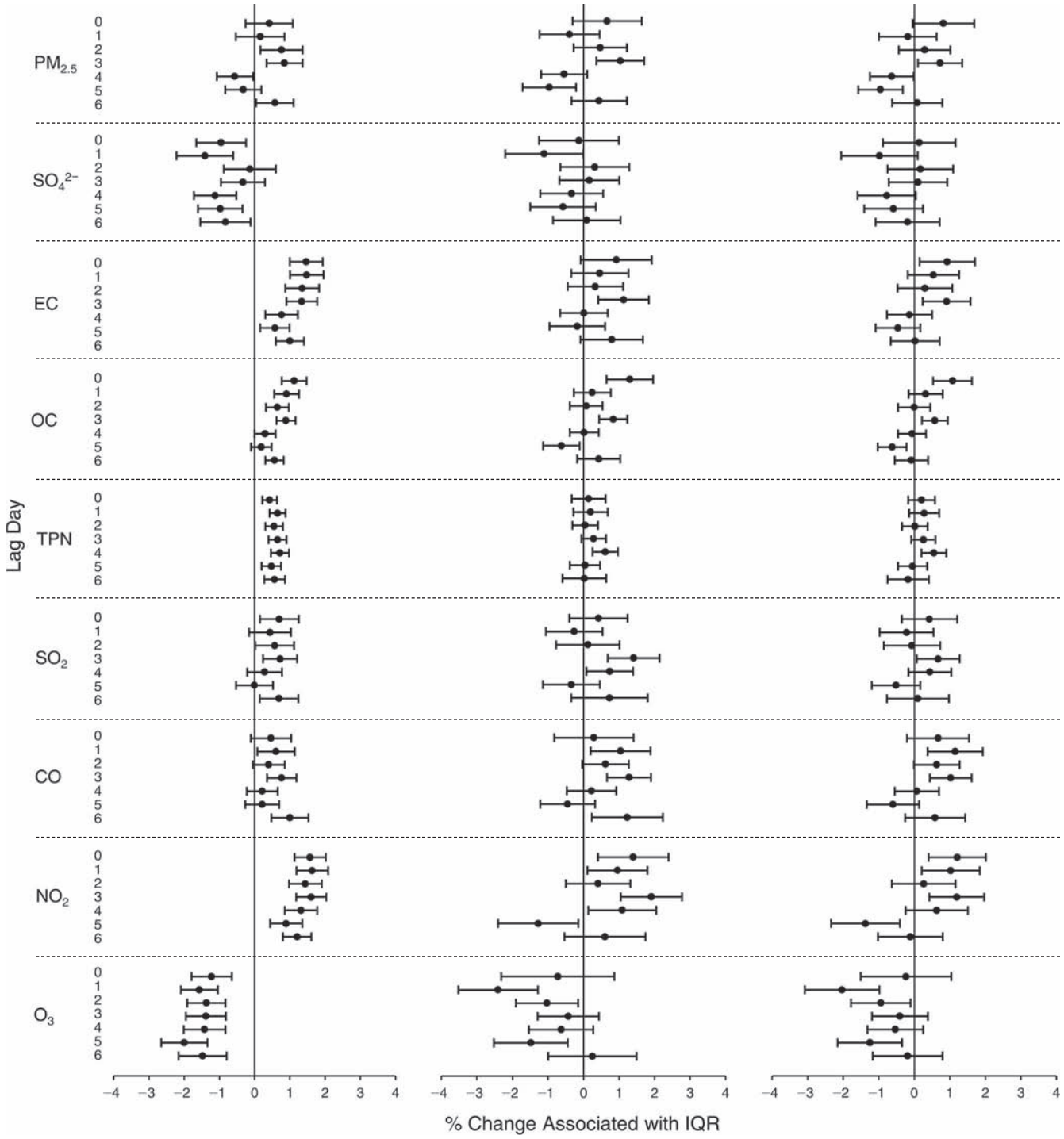


Figure K.11. Estimated means and 95% CIs for the percent change in SBP associated with one IQR increase in pollutant concentration. Left: results without any adjustment for temperature or RH; **Middle:** results with full adjustments; **Right:** results with adjustments for temperature and RH having statistically significant effects on biomarkers.

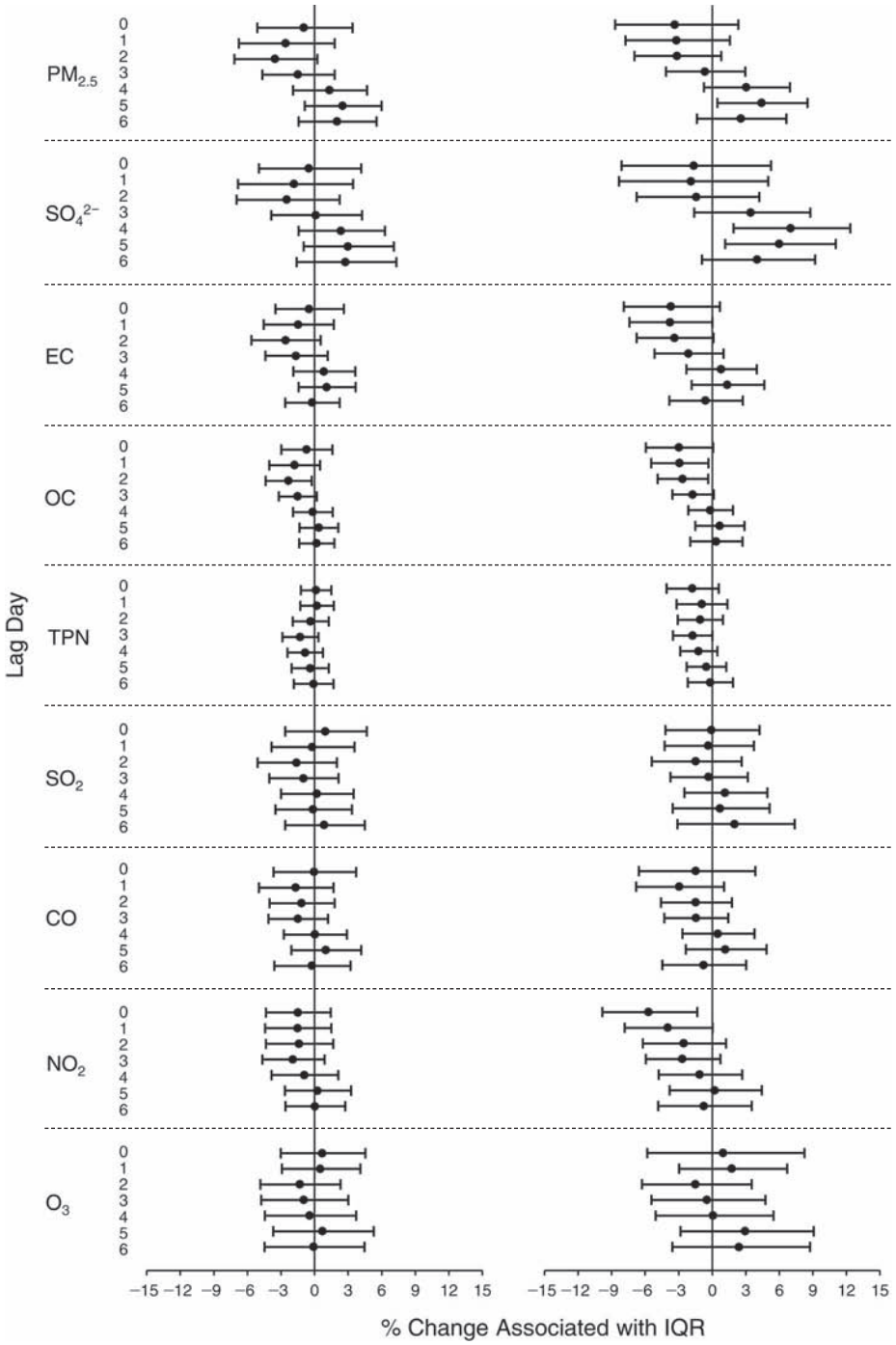


Figure K.12. Estimated means and 95% CIs for the percent change in SDNN (HRV) associated with one IQR increase in pollutant concentration. Left: results without any adjustment for temperature or RH; **Right:** results with full adjustments.

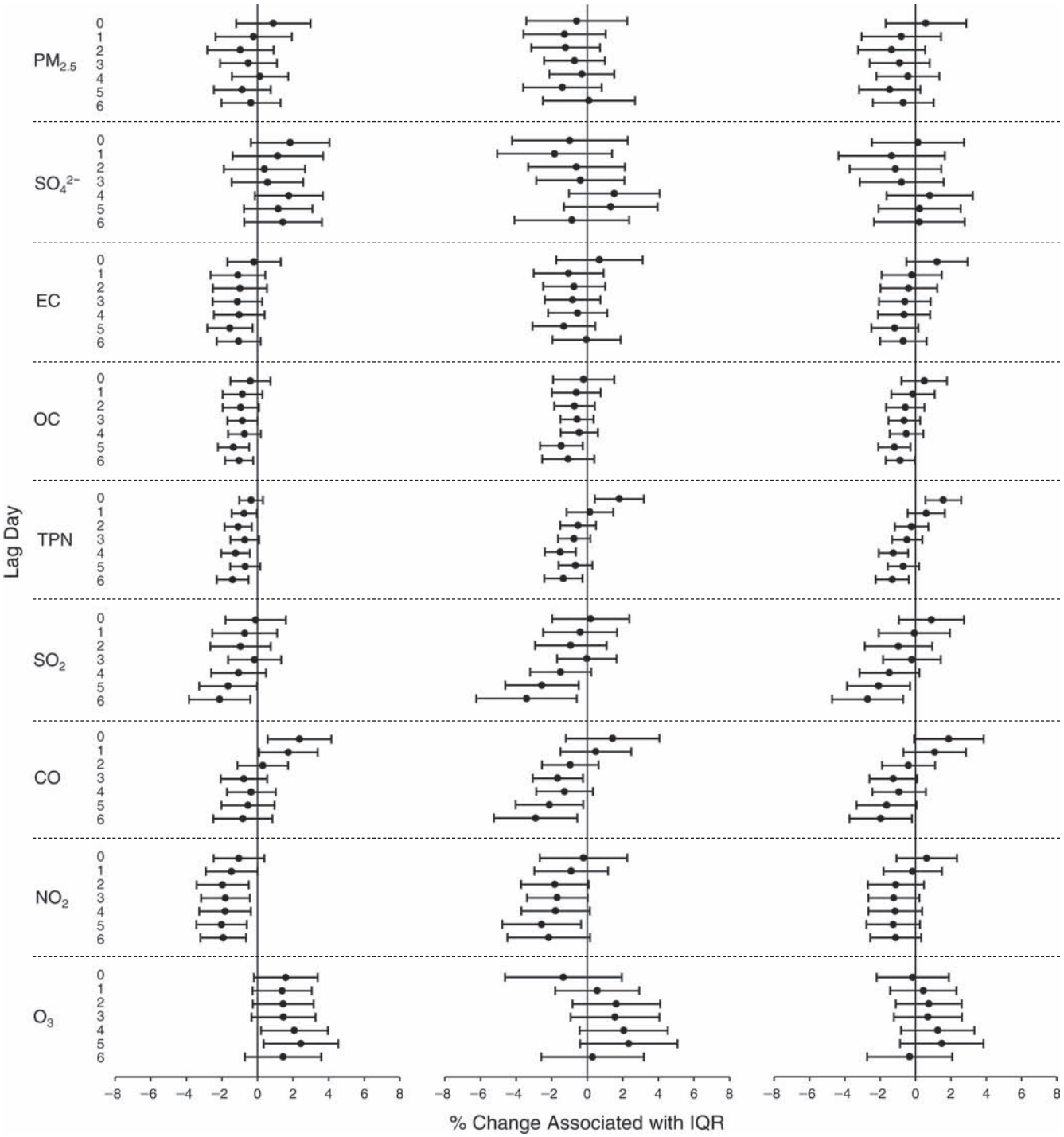


Figure K.13. Estimated means and 95% CIs for the percent change in WBC count associated with one IQR increase in pollutant concentration. Left: results without any adjustment for temperature or RH; **Middle:** results with full adjustments; **Right:** results with adjustments for temperature and RH having statistically significant effects on biomarkers.

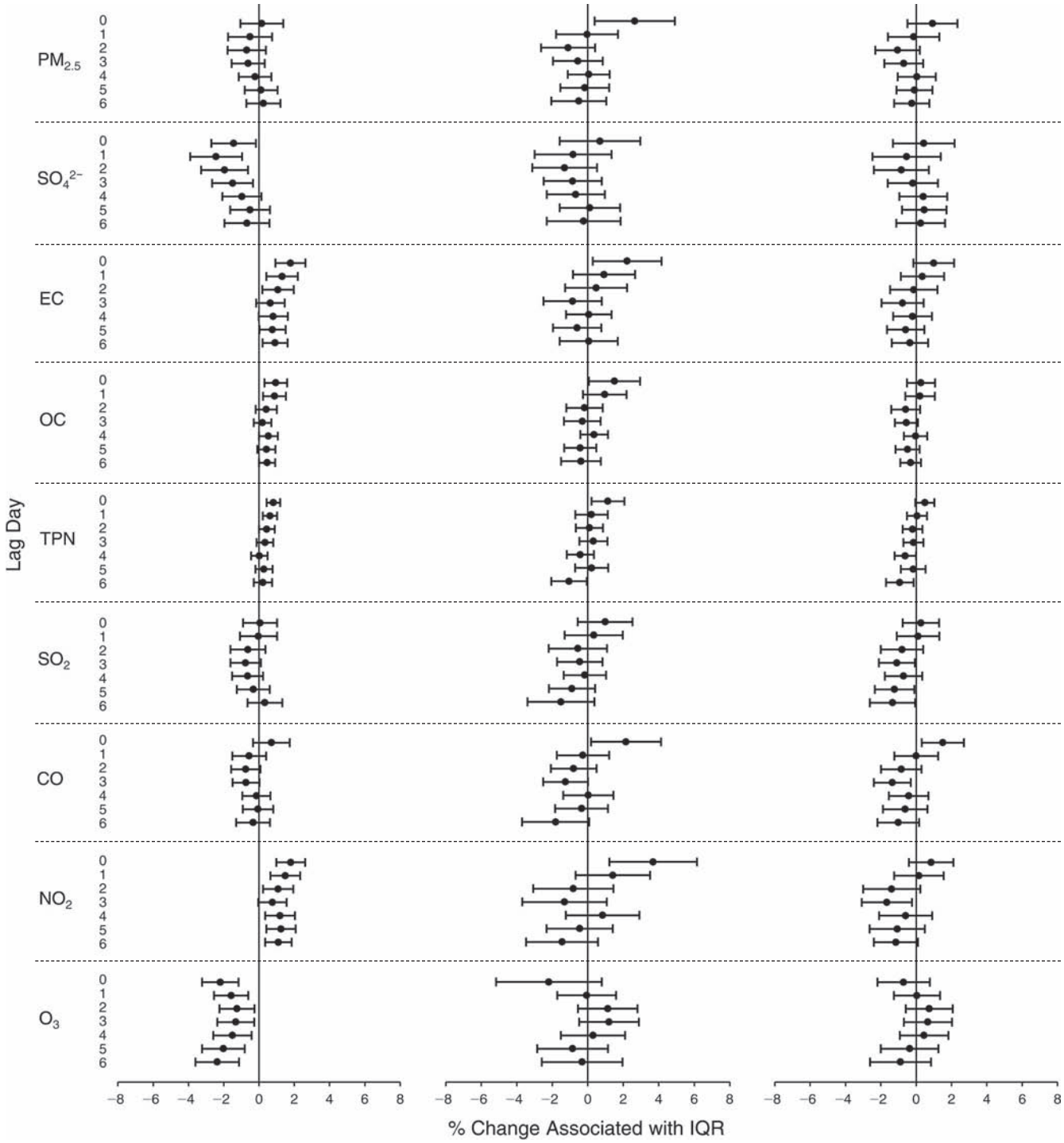


Figure K.14. Estimated means and 95% CIs for the percent change in percentage of neutrophils associated with one IQR increase in pollutant concentration. Left: results without any adjustment for temperature or RH; **Middle:** results with full adjustments; **Right:** results with adjustments for temperature and RH having statistically significant effects on biomarkers.

APPENDIX L. Percent Change in Biomarkers Associated with One IQR Increase in Pollutant Concentration, Controlling for Several Factors and Excluding Rainy Days

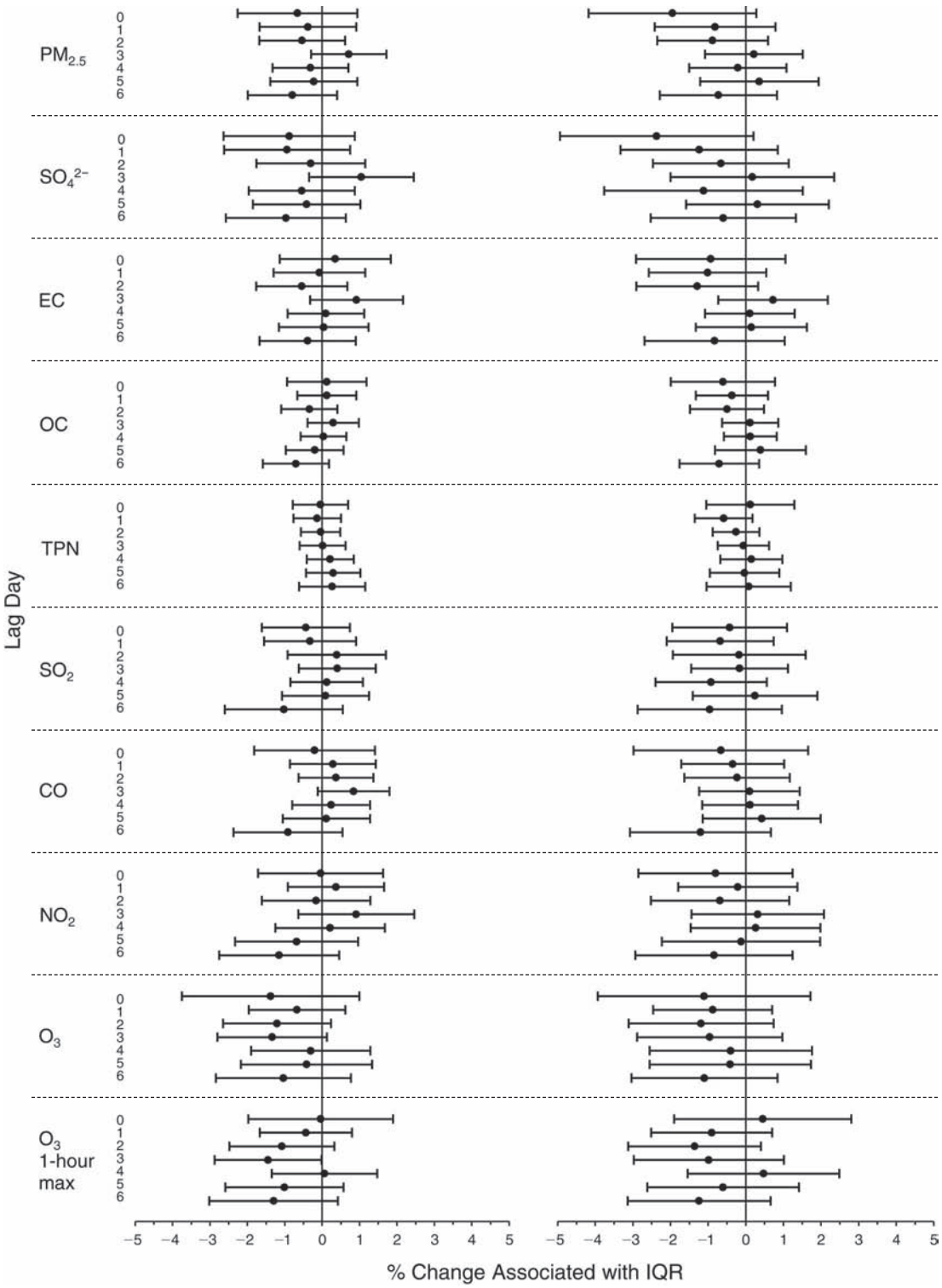


Figure L.1. Estimated means and 95% CIs for the percent change in DBP associated with one IQR increase in pollutant concentration, controlling for temperature (df = 3), RH (df = 3), 7-day moving average for temperature (df = 3), 5-day moving average for RH (df = 3), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

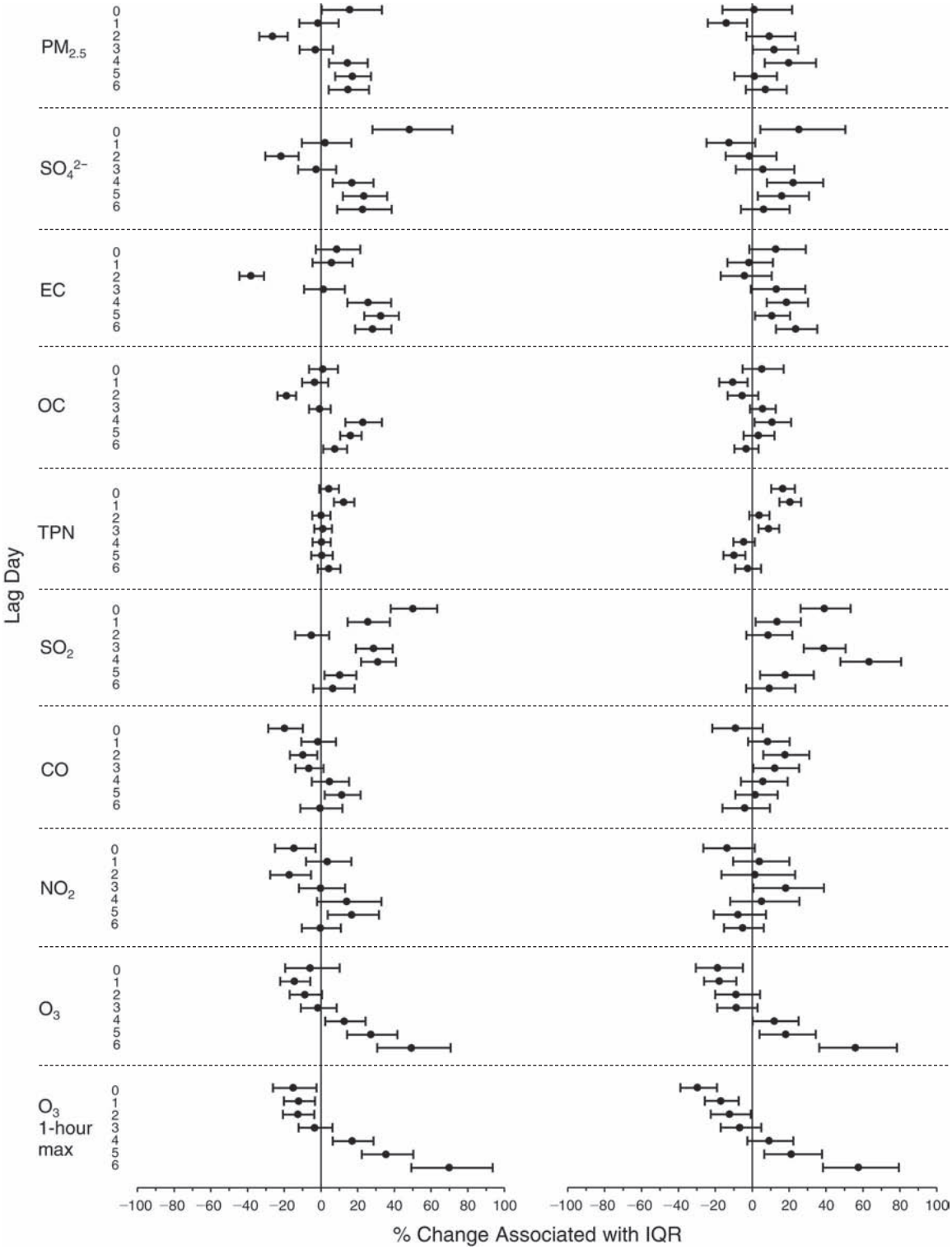


Figure L.2. Estimated means and 95% CIs for the percent change in EBC nitrate associated with one IQR increase in pollutant concentration, controlling for temperature (df = 2), RH (df = 1), 7-day moving average for temperature (df = 3), 7-day moving average for RH (df = 3), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

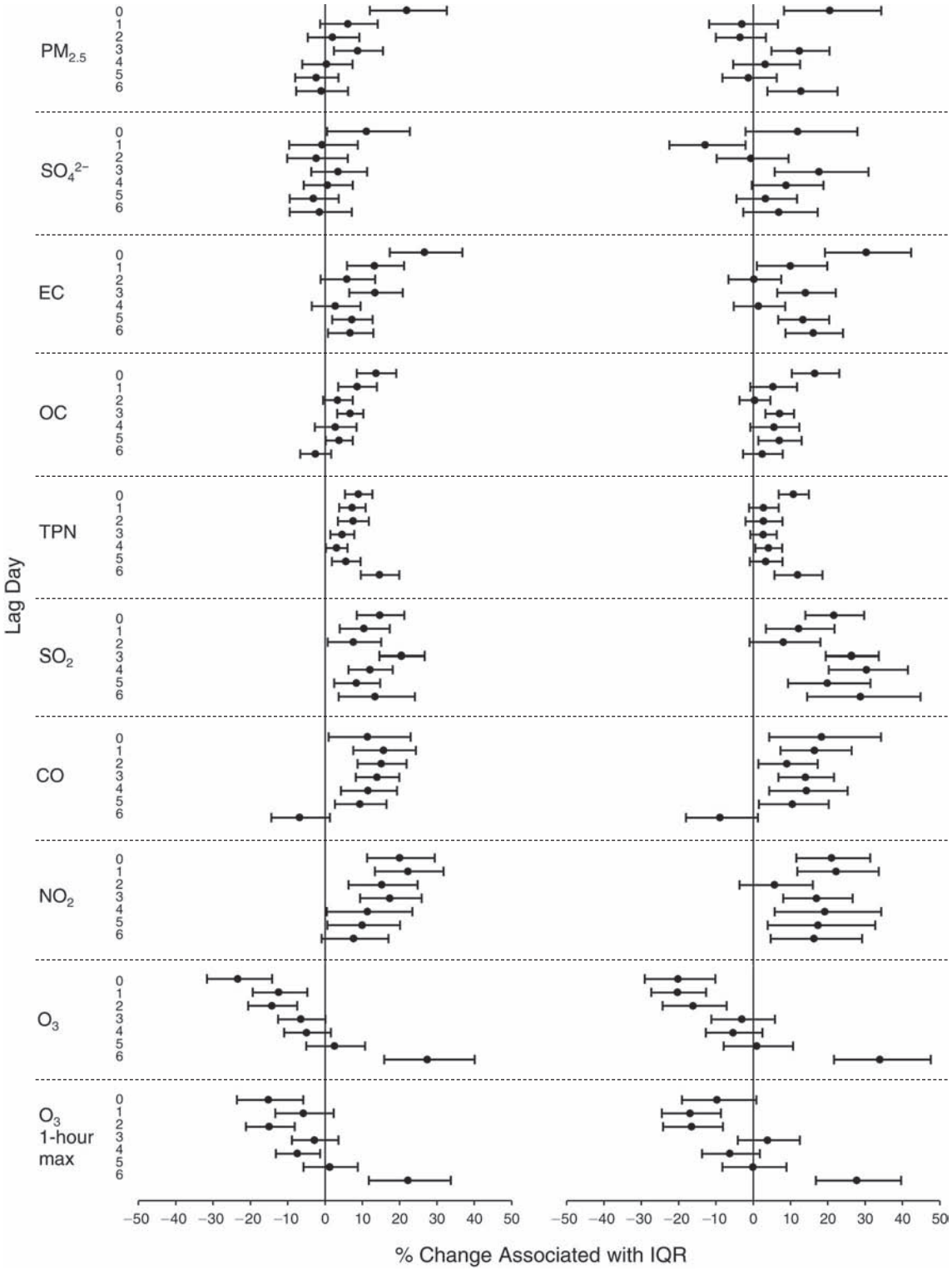


Figure L.3. Estimated means and 95% CIs for the percent change in EBC nitrite associated with one IQR increase in pollutant concentration, controlling for temperature (df = 2), RH (df = 1), 7-day moving average for temperature (df = 3), 3-day moving average for RH (df = 3), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

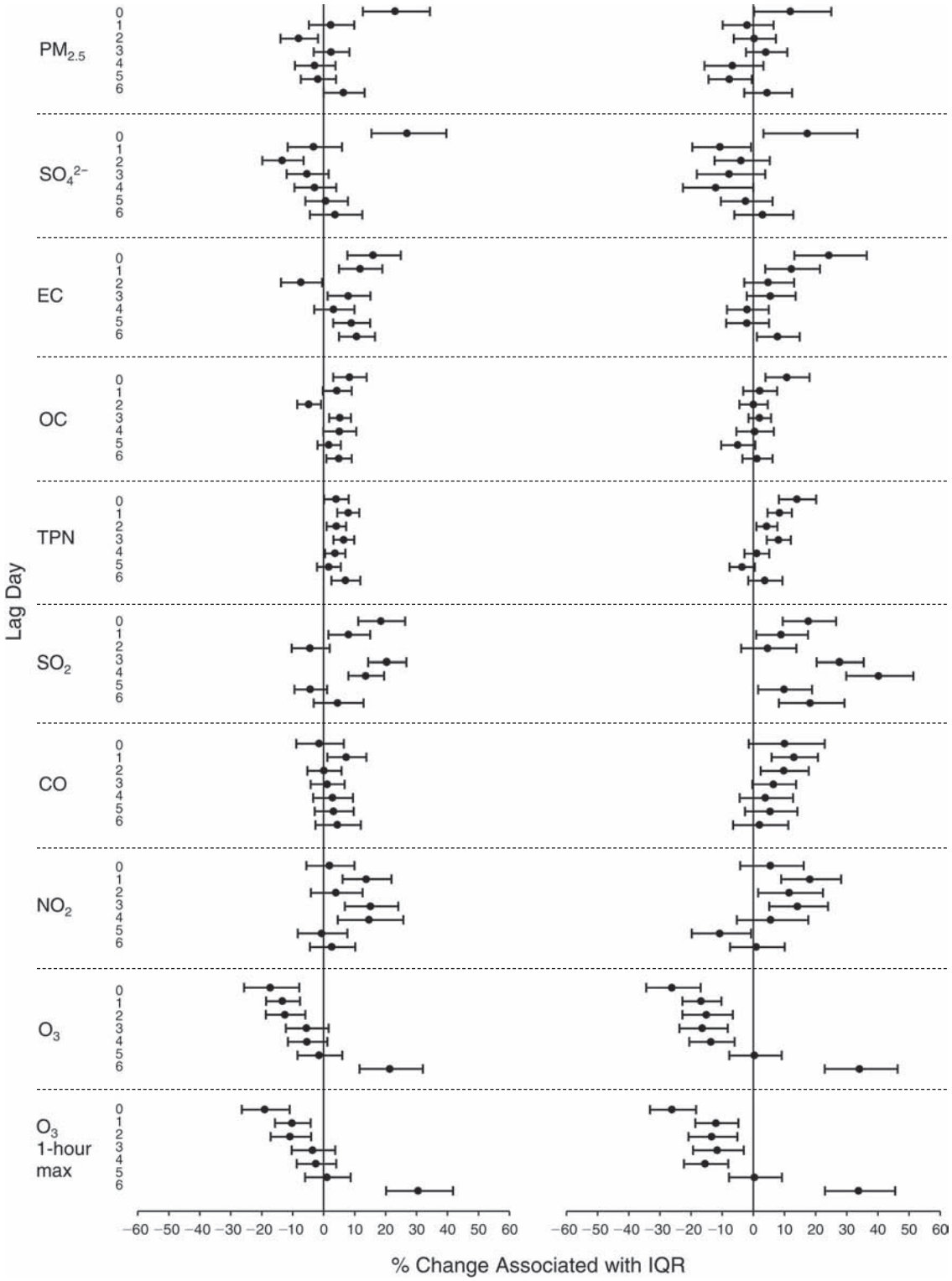


Figure L.4. Estimated means and 95% CIs for the percent change in EBC nitrite+nitrate associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 3), 7-day moving average for temperature (df = 3), 5-day moving average for RH (df = 3), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

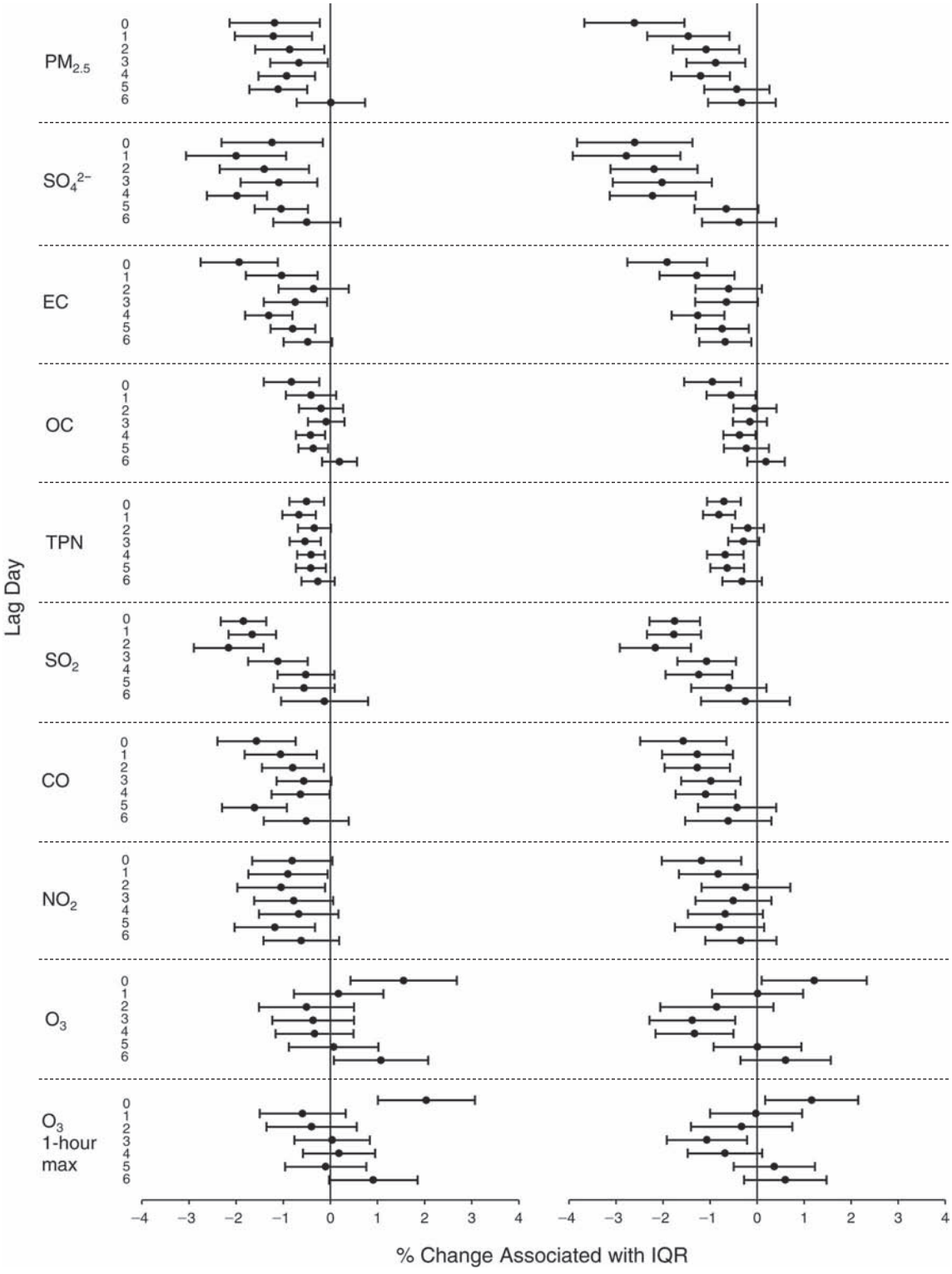


Figure L.5. Estimated means and 95% CIs for the percent change in EBC pH associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 6-day moving average for temperature (df = 3), 3-day moving average for RH (df = 1), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

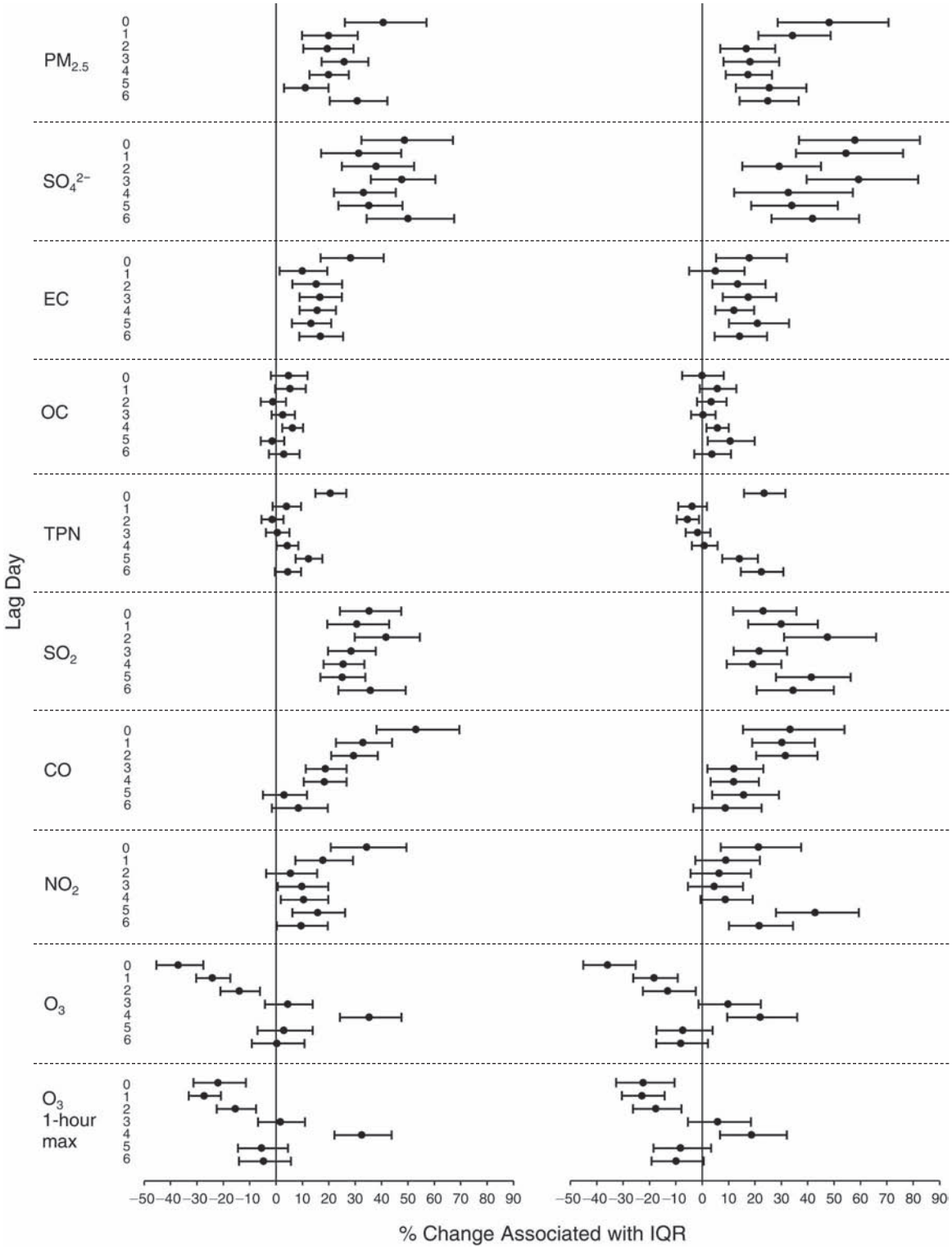


Figure L.6. Estimated means and 95% CIs for the percent change in FeNO associated with one IQR increase in pollutant concentration, controlling for temperature (df = 2), RH (df = 3), 7-day moving average of temperature (df = 2), 7-day moving average of RH (df = 3), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

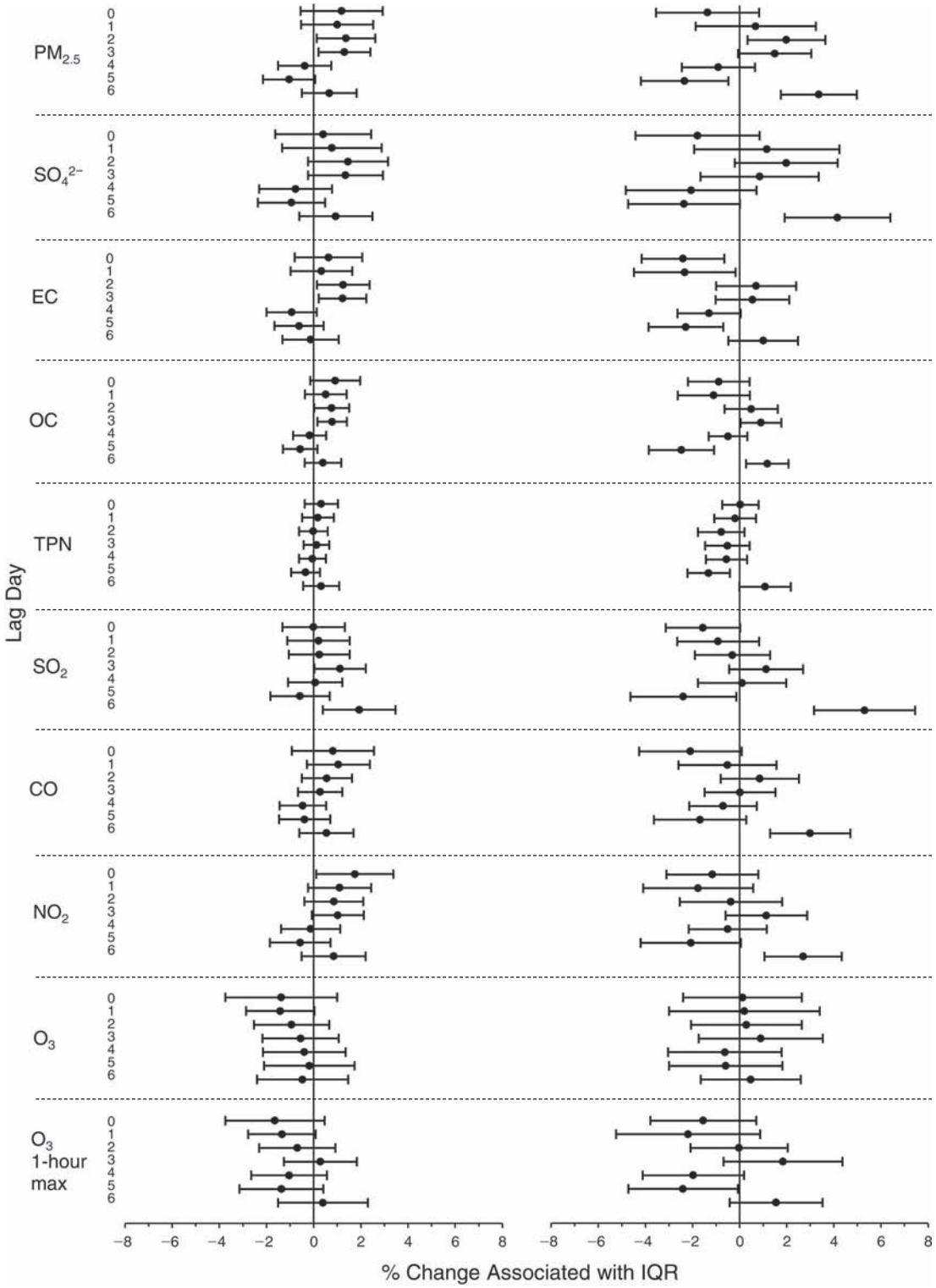


Figure L.7. Estimated means and 95% CIs for the percent change in fibrinogen associated with one IQR increase in pollutant concentration, controlling for temperature (df = 3), RH (df = 1), 6-day moving average for temperature (df = 1), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

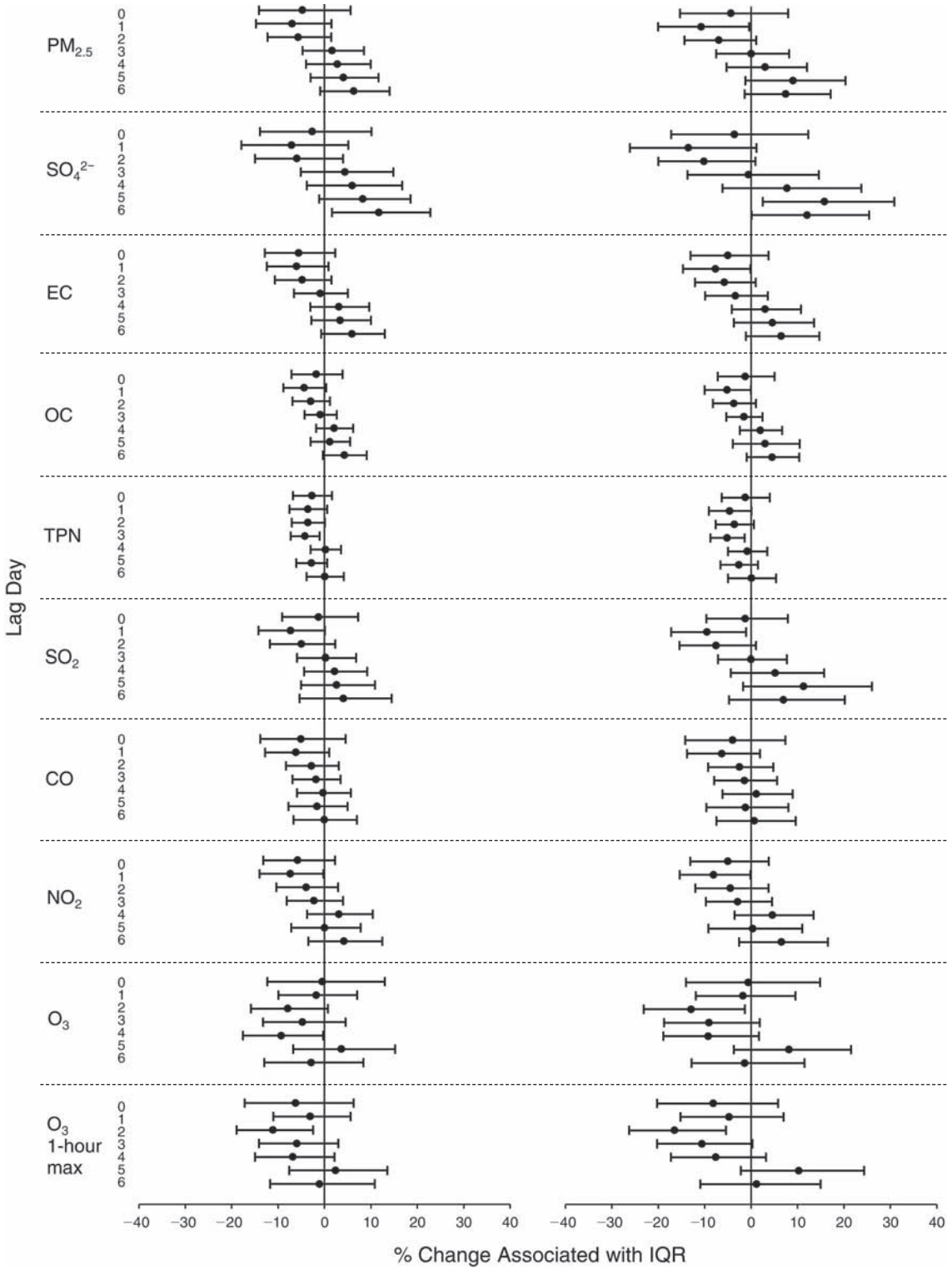


Figure L.8. Estimated means and 95% CIs for the percent change in HF (HRV) associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 7-day moving average for temperature (df = 1), and sex. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

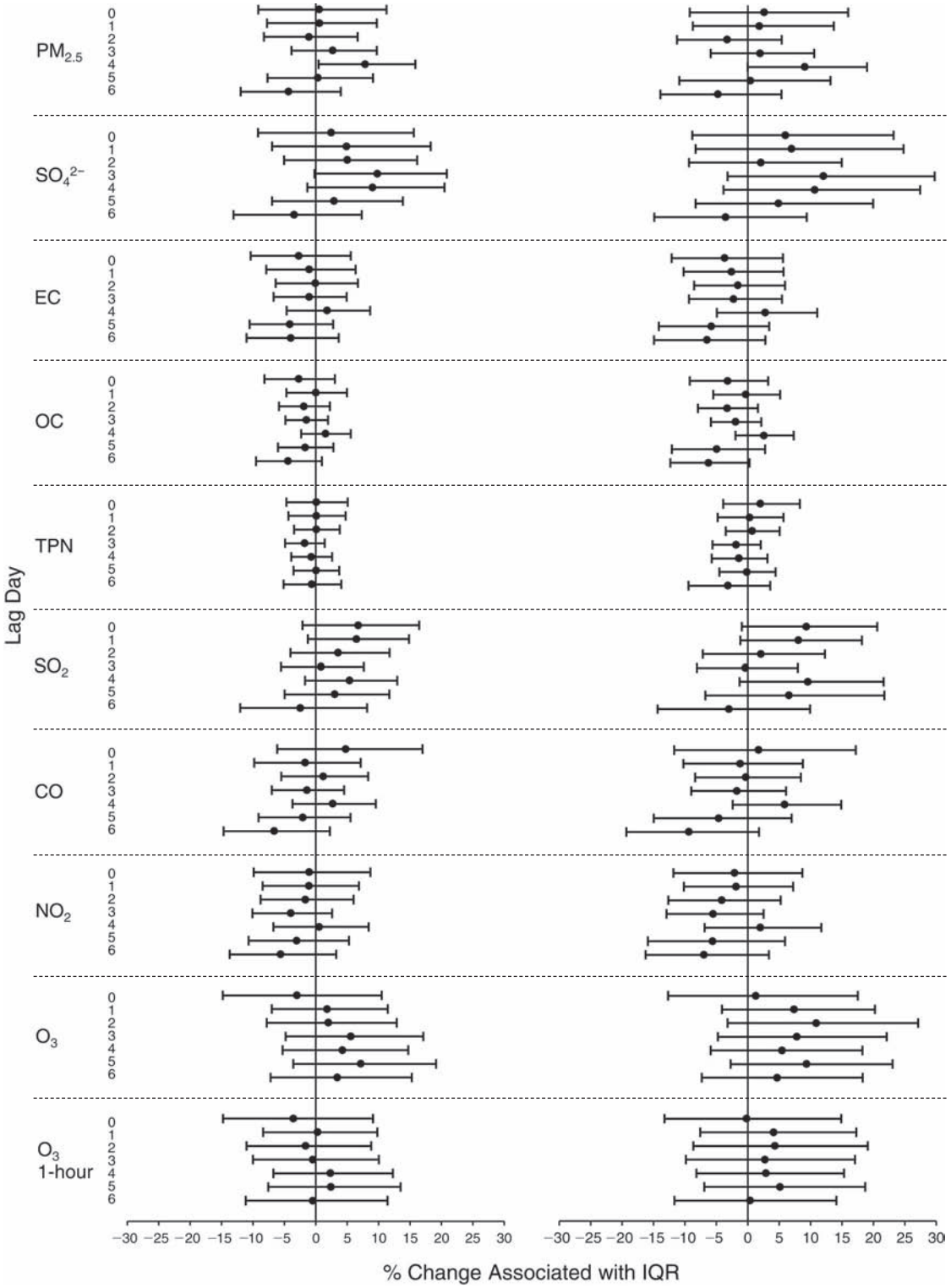


Figure L.9. Estimated means and 95% CIs for the percent change in LF (HRV) associated with one IQR increase in pollutant concentration, controlling for temperature (df = 2), RH (df = 1), 7-day moving average for temperature (df = 1), 5-day moving average for RH (df = 1), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

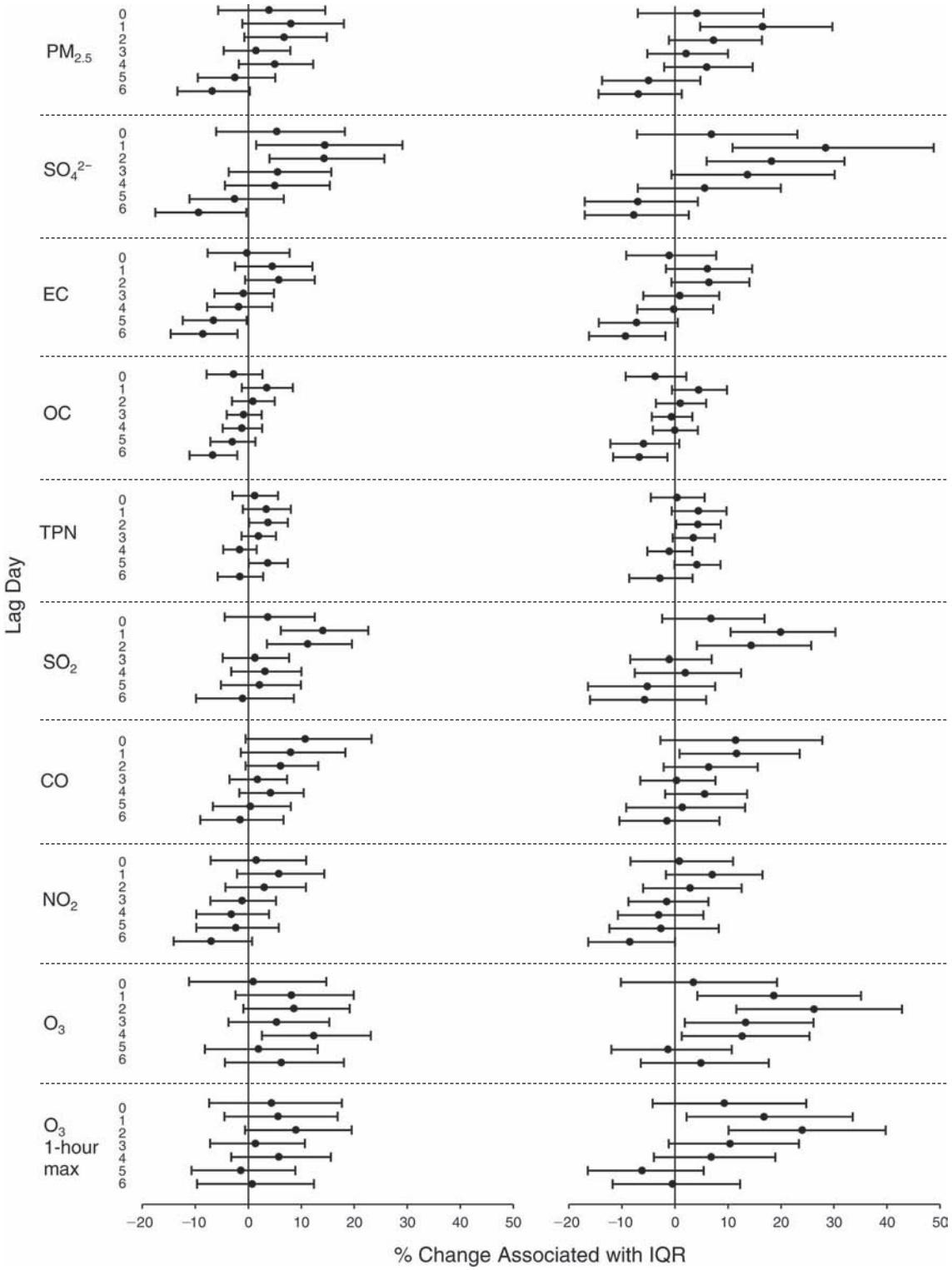


Figure L.10. Estimated means and 95% CIs for the percent change in LF/HF (HRV) associated with one IQR increase in pollutant concentration, controlling for temperature (df = 2), RH (df = 1), 7-day moving average for temperature (df = 1), 2-day moving average for RH (df = 1), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

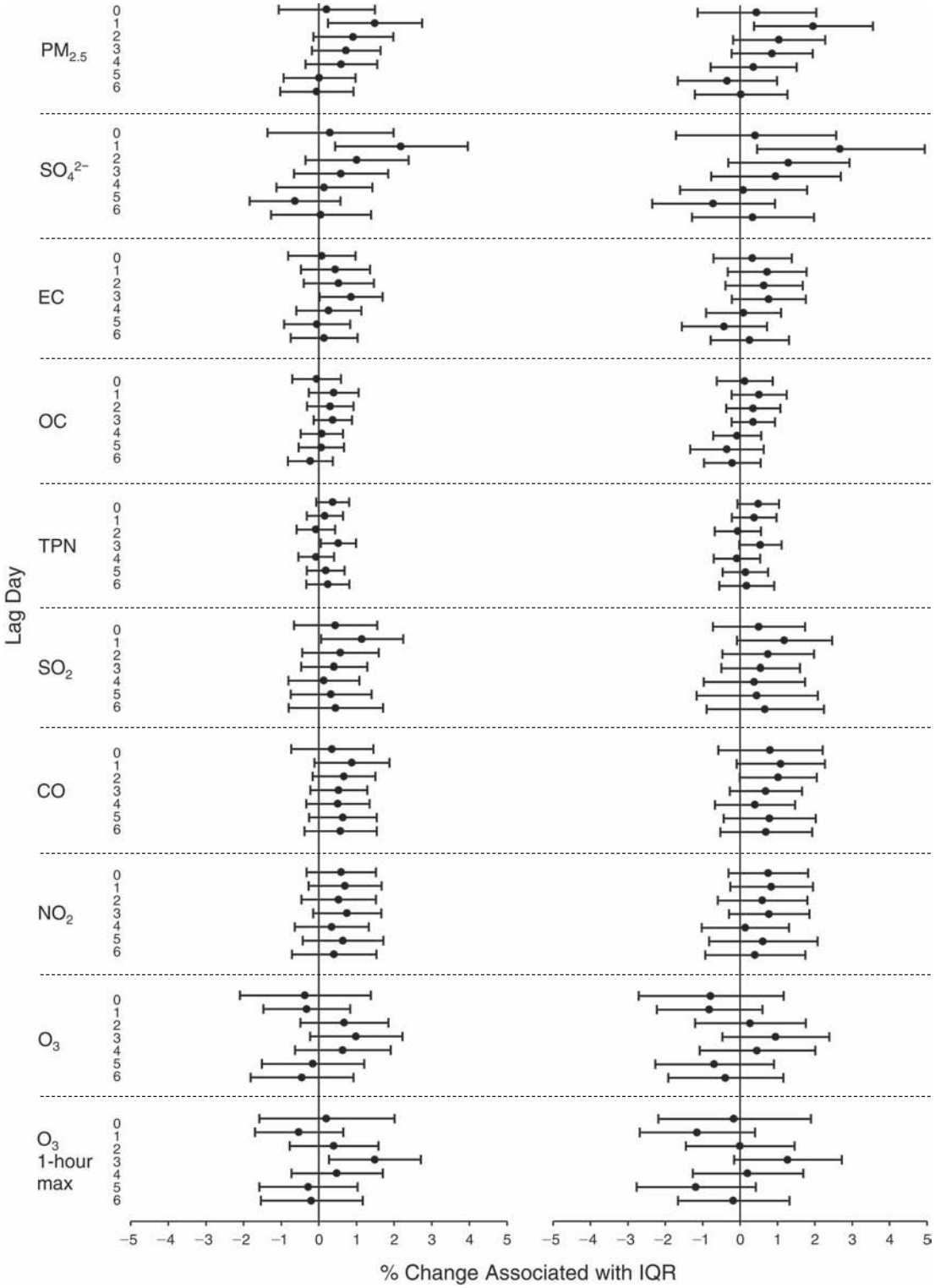


Figure L.11. Estimated means and 95% CIs for the percent change in HR associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

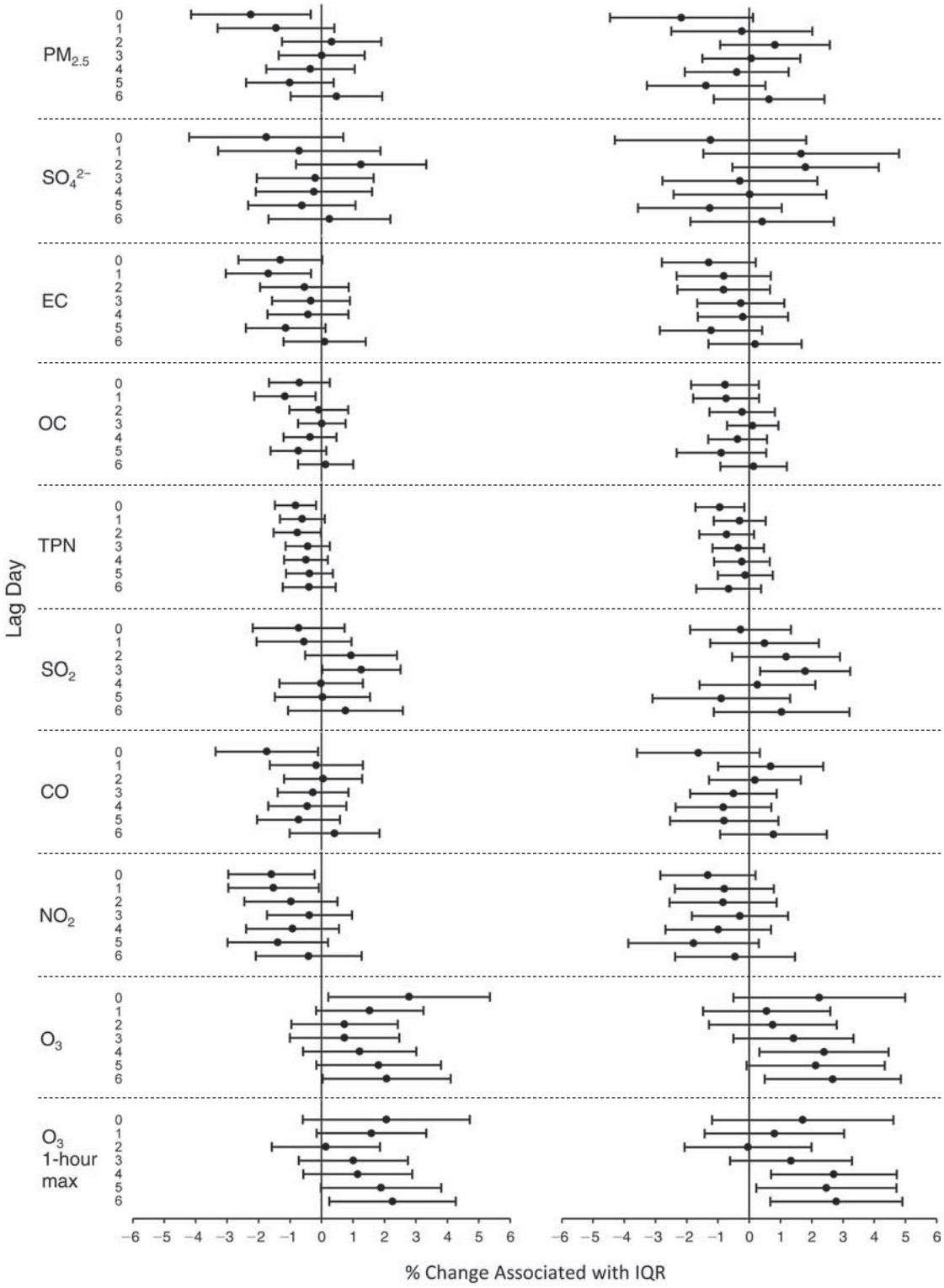


Figure L.12. Estimated means and 95% CIs for the percent change in lymphocyte count associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

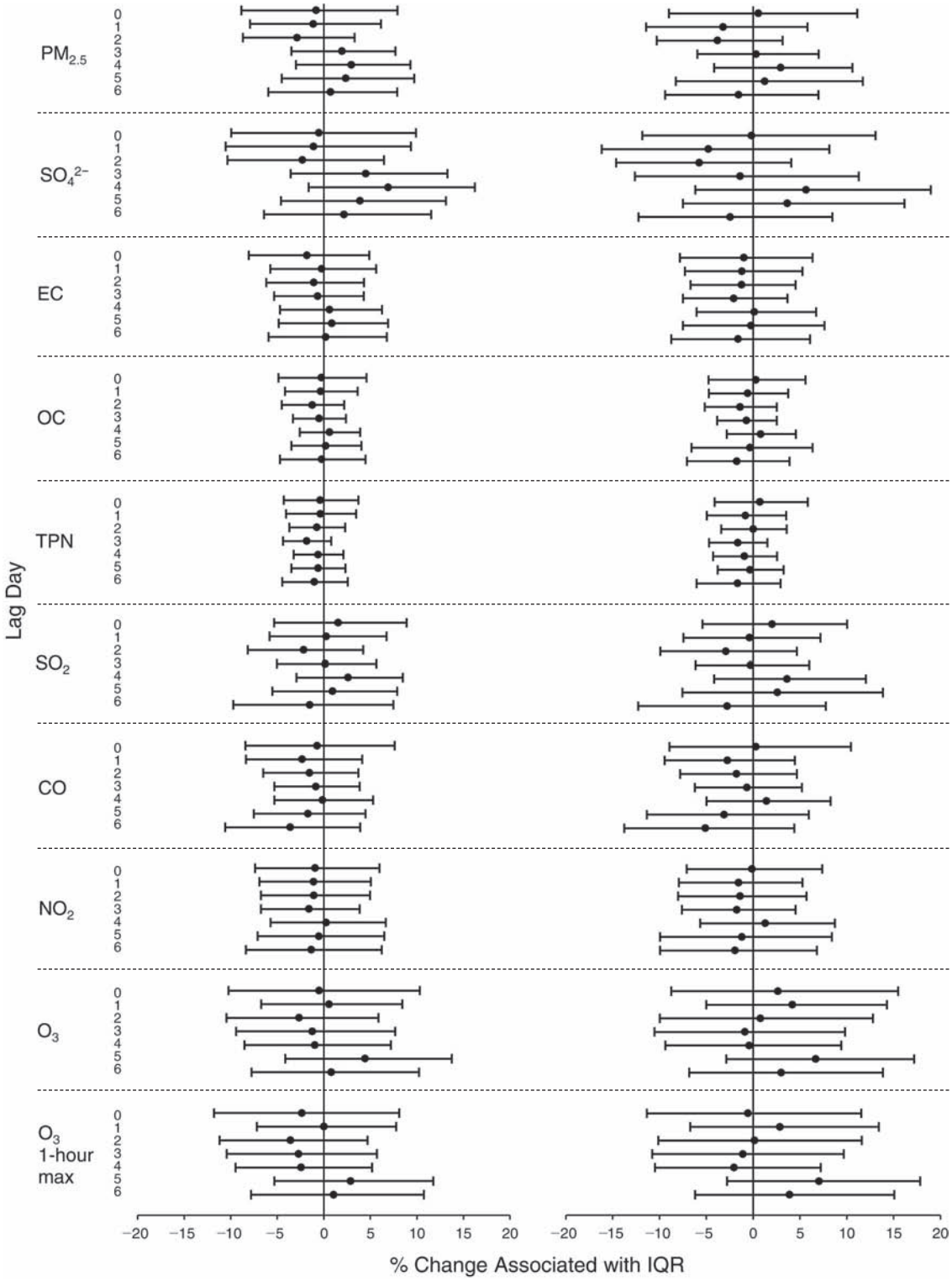


Figure L.13. Estimated means and 95% CIs for the percent change in total power (HRV) associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 7-day moving average for temperature (df = 1), 5-day moving average for RH (df = 1), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

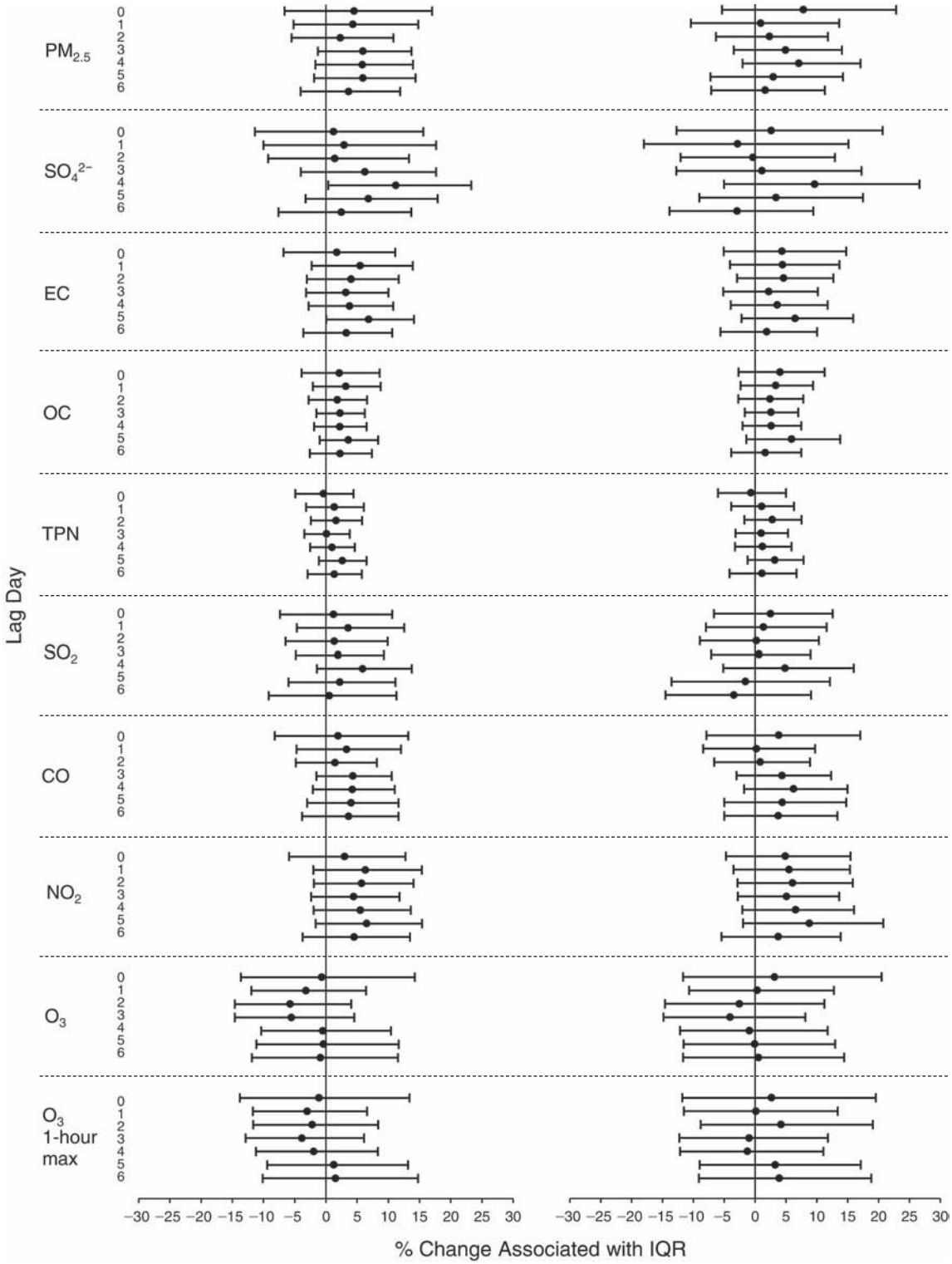


Figure L.14. Estimated means and 95% CIs for the percent change in VLF (HRV) associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 7-day moving average for temperature (df = 1), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

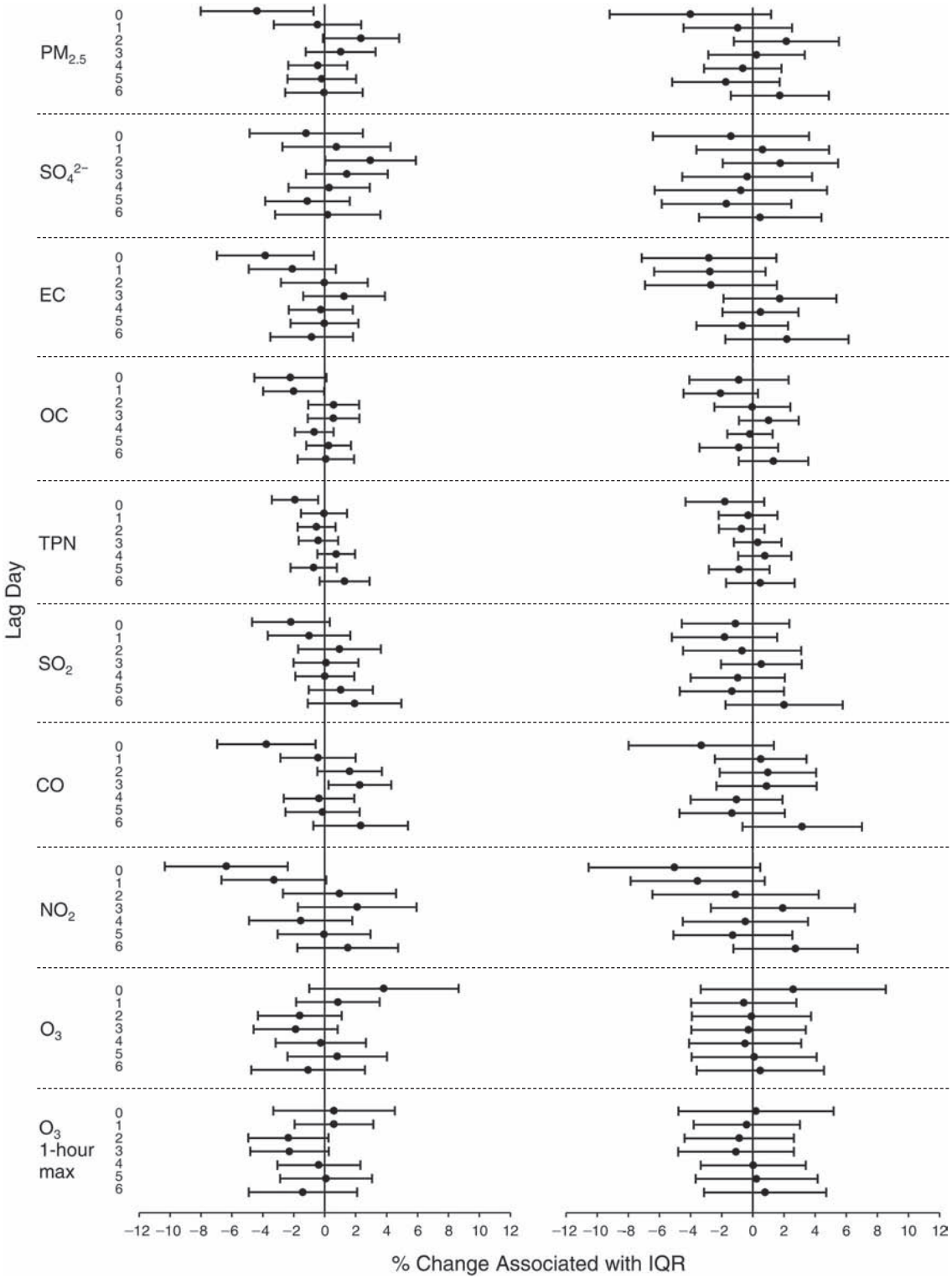


Figure L.15. Estimated means and 95% CIs for the percent change in percentage of lymphocytes associated with one IQR increase in pollutant concentration, controlling for temperature (df = 3), RH (df = 3), 7-day moving average for temperature (df = 3), 7-day moving average for RH (df = 3), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

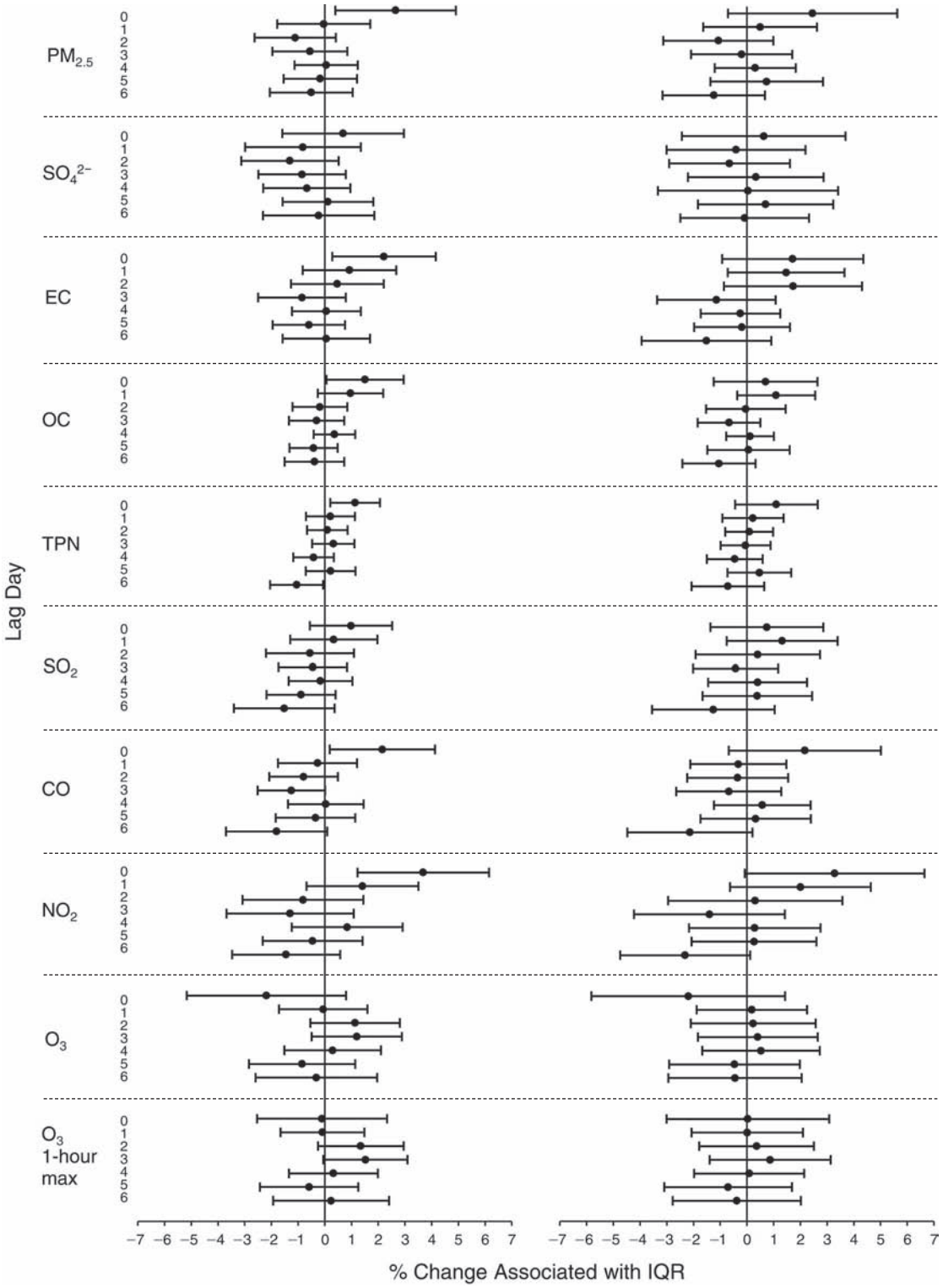


Figure L.16. Estimated means and 95% CIs for the percent change in neutrophils associated with one IQR increase in pollutant concentration, controlling for temperature (df = 3), RH (df = 3), 7-day moving average for temperature (df = 3), 2-day moving average for RH (df = 3), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

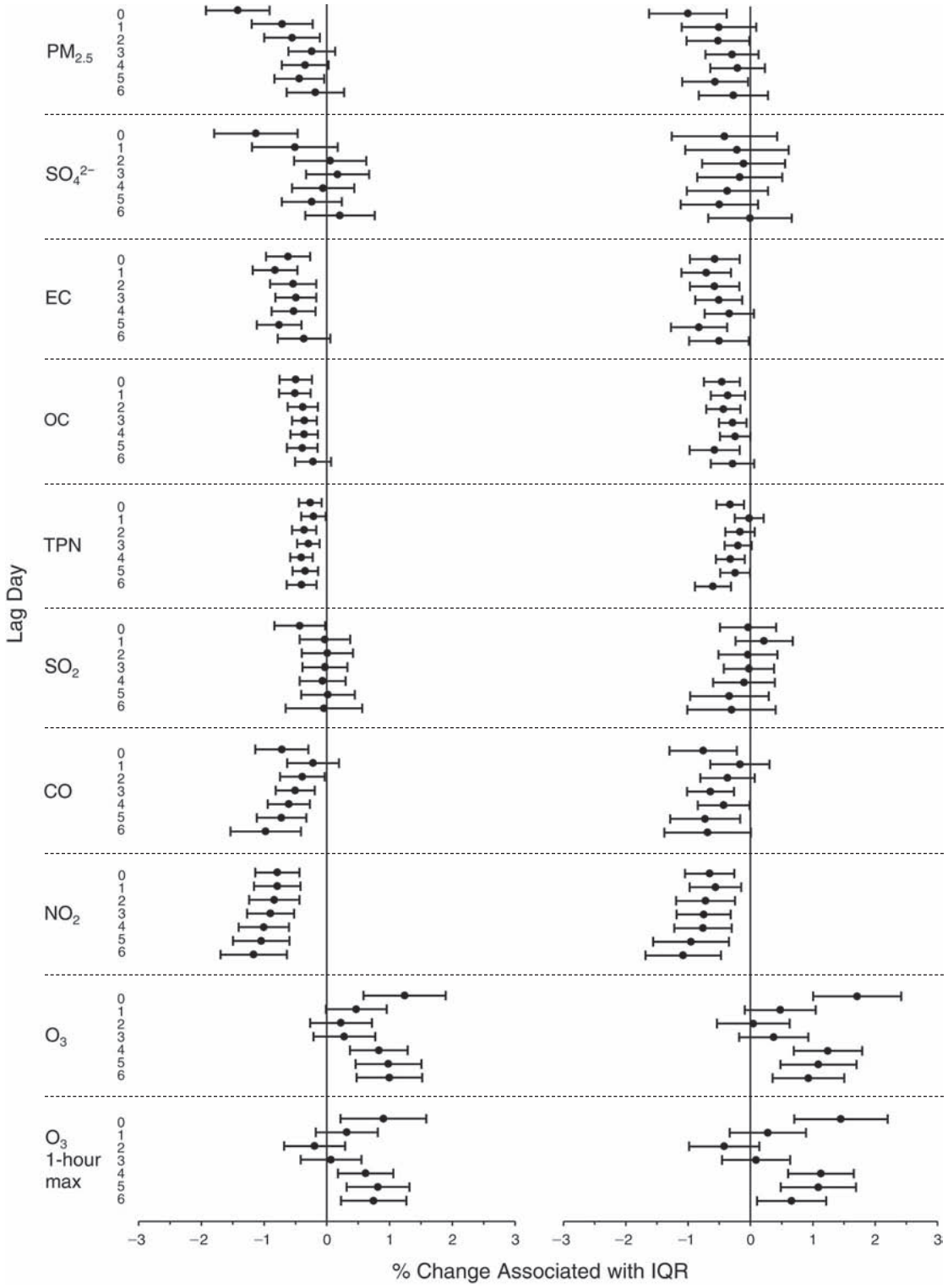


Figure L.17. Estimated means and 95% CIs for the percent change in RBC count associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 4-day moving average for RH (df = 1), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

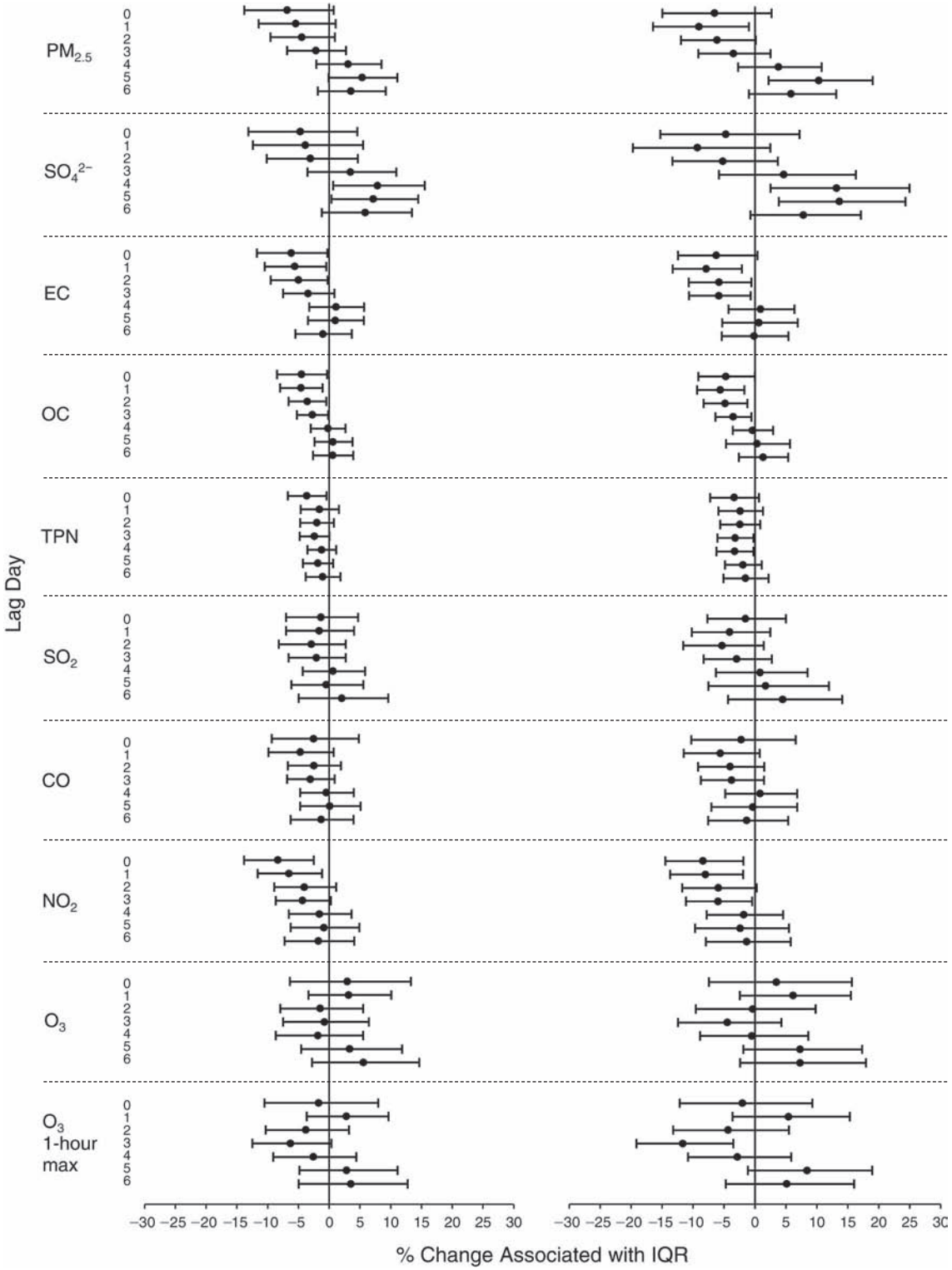


Figure L.18. Estimated means and 95% CIs for the percent change in rMSSD (HRV) associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 7-day moving average for temperature (df = 1), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

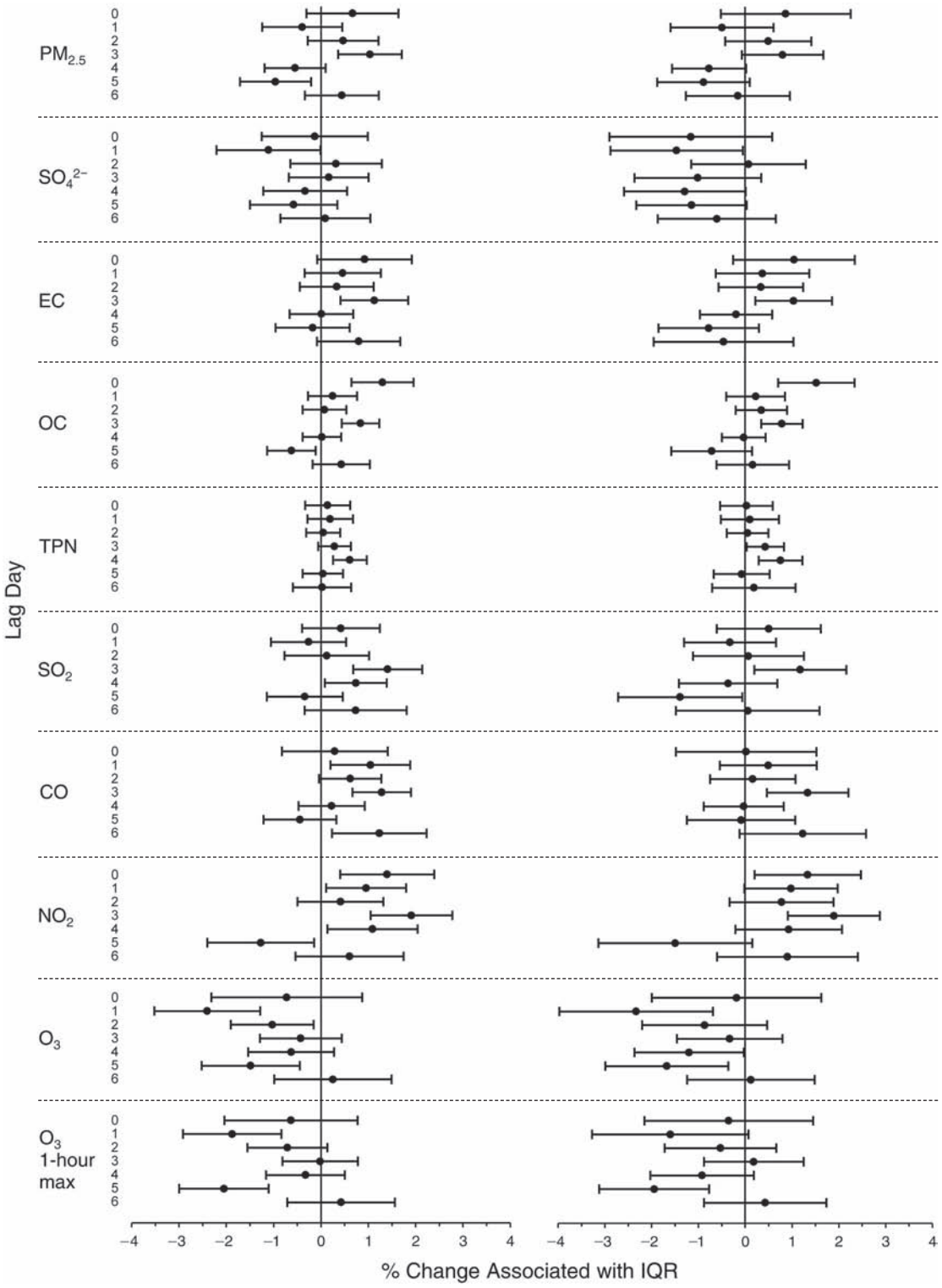


Figure L.19. Estimated means and 95% CIs for the percent change in SBP associated with one IQR increase in pollutant concentration, controlling for temperature (df = 3), RH (df = 2), 7-day moving average for temperature (df = 3), 2-day moving average for RH (df = 3), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

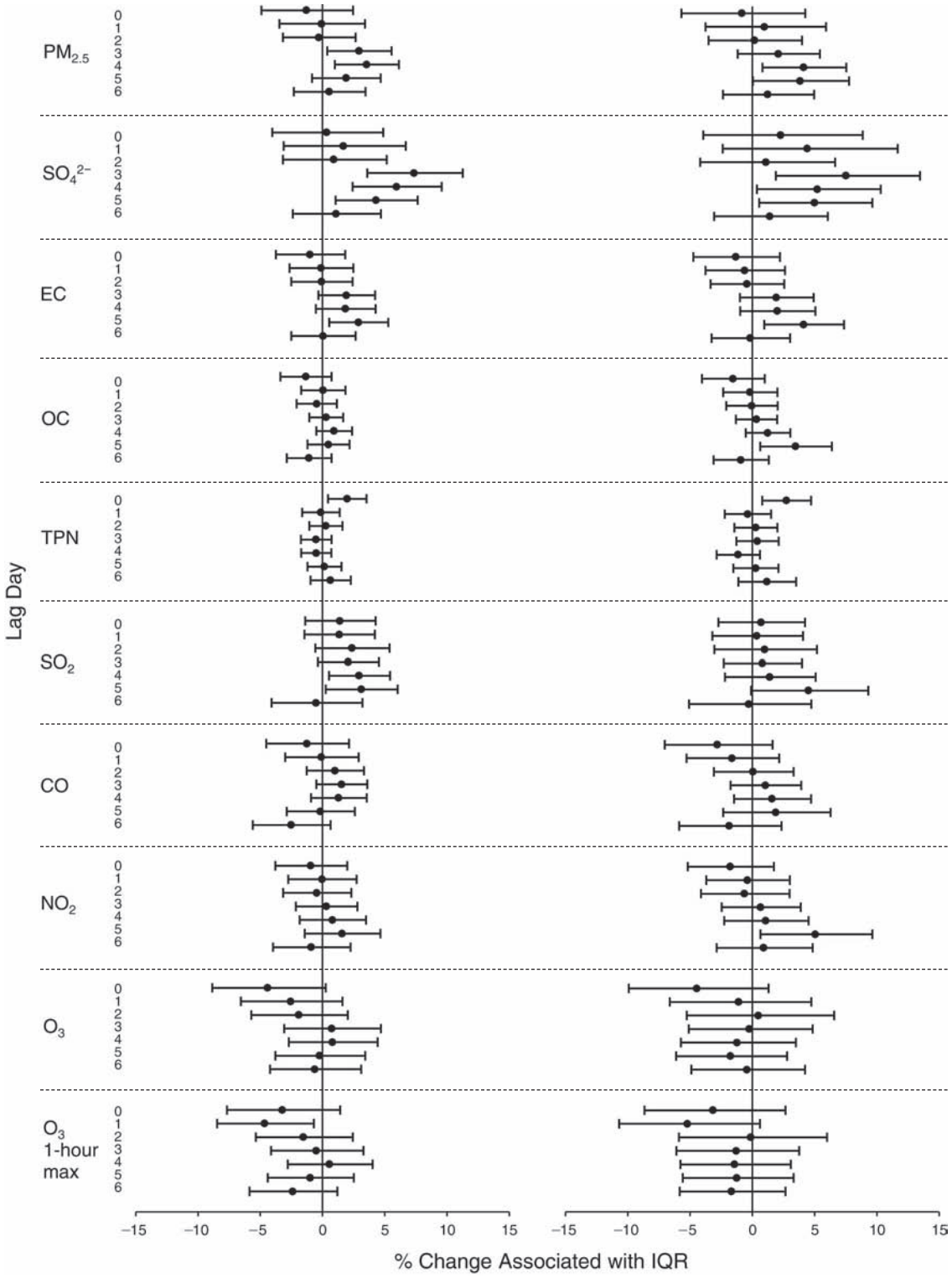


Figure L.20. Estimated means and 95% CIs for the percent change in sCD40L associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 5-day moving average for temperature (df = 1), 2-day moving average for RH (df = 1), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

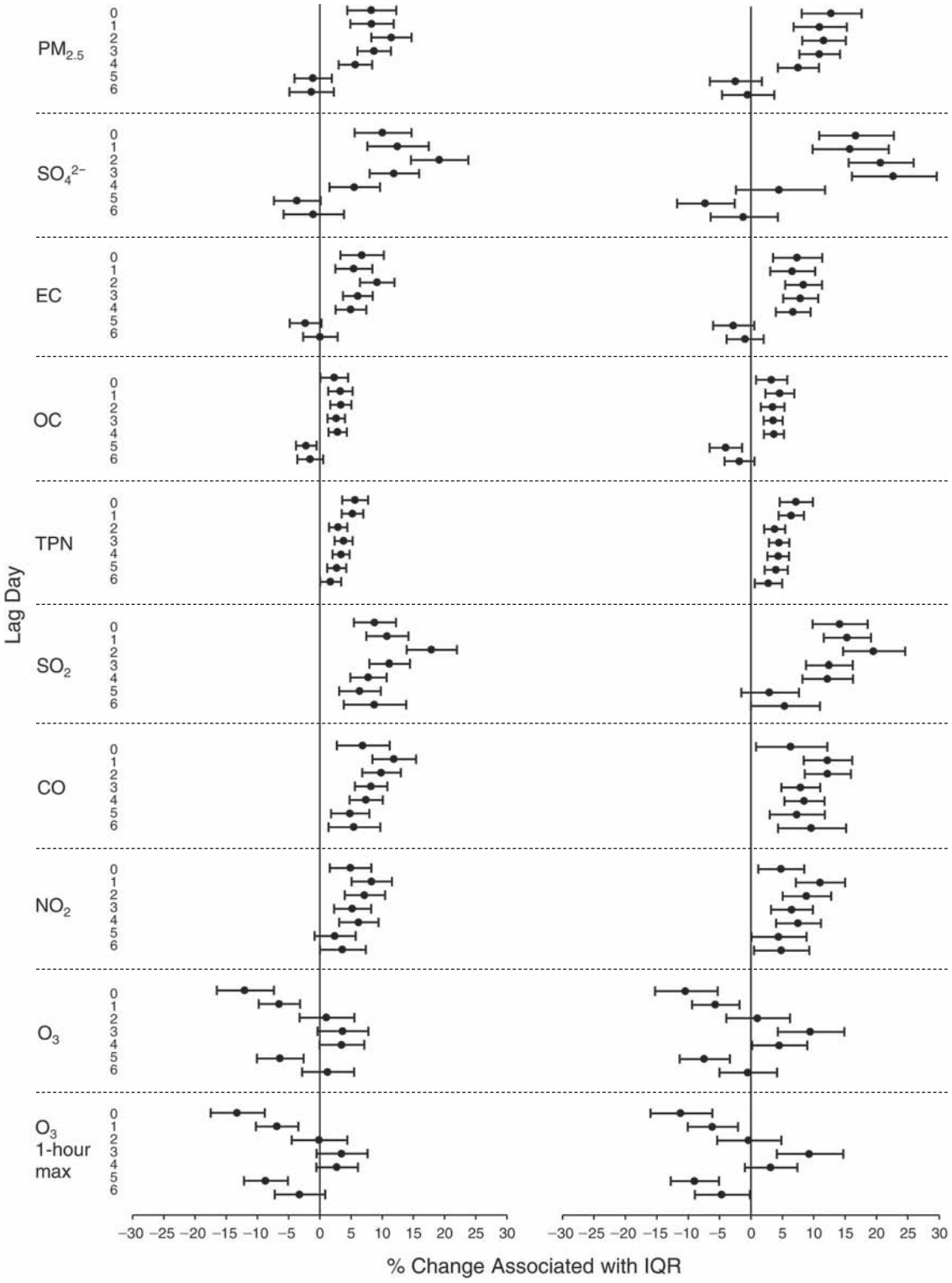


Figure L.21. Estimated means and 95% CIs for the percent change in sCD62P associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 3), 7-day moving average for temperature (df = 2), 4-day moving average for RH (df = 2), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

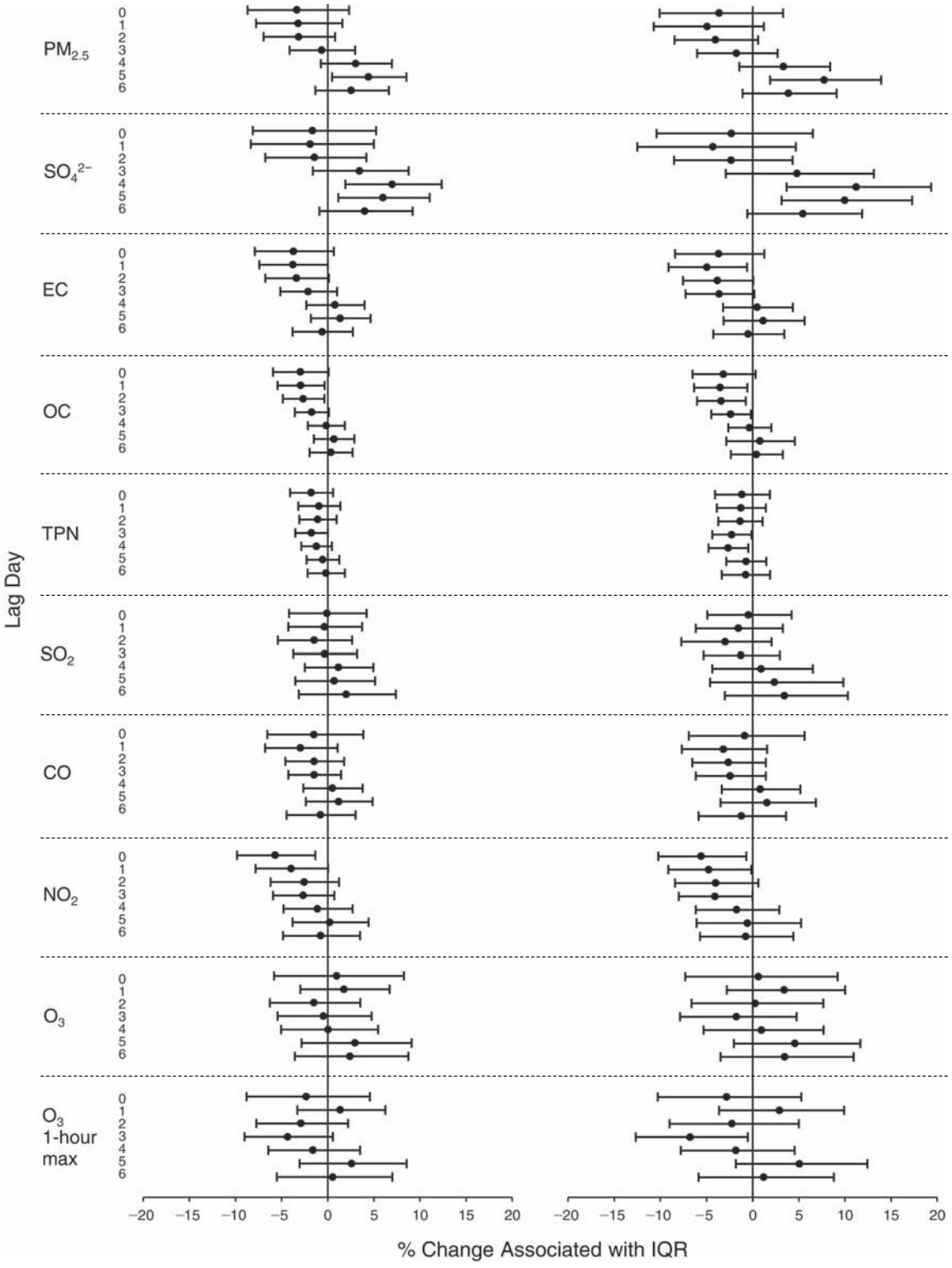


Figure L.22. Estimated means and 95% CIs for the percent change in SDNN associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 7-day moving average for temperature (df = 1), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

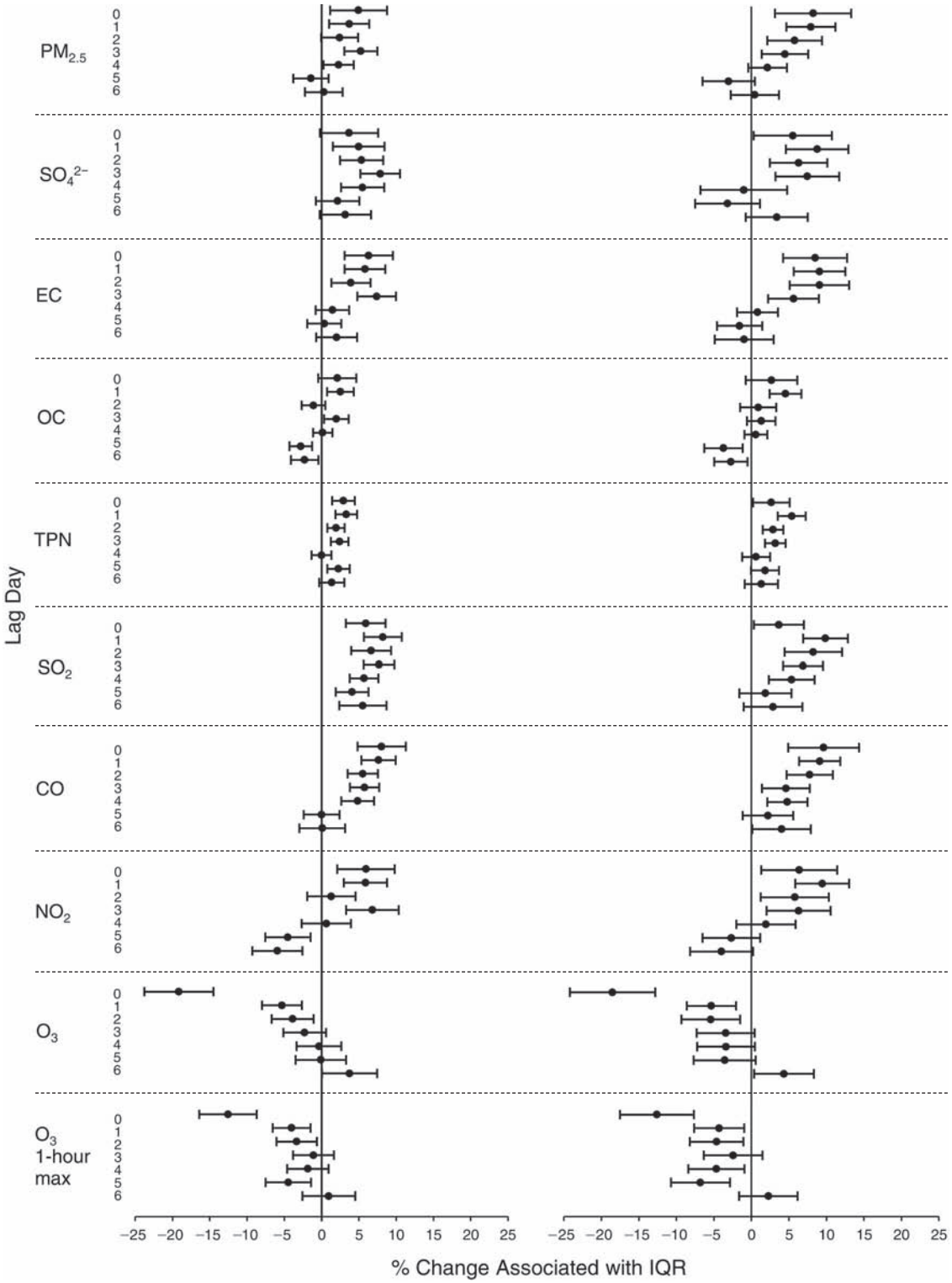


Figure L.23. Estimated means and 95% CIs for the percent change in vWF associated with one IQR increase in pollutant concentration, controlling for temperature (df = 3), RH (df = 3), 7-day moving average for temperature (df = 3), 6-day moving average for RH (df = 3), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

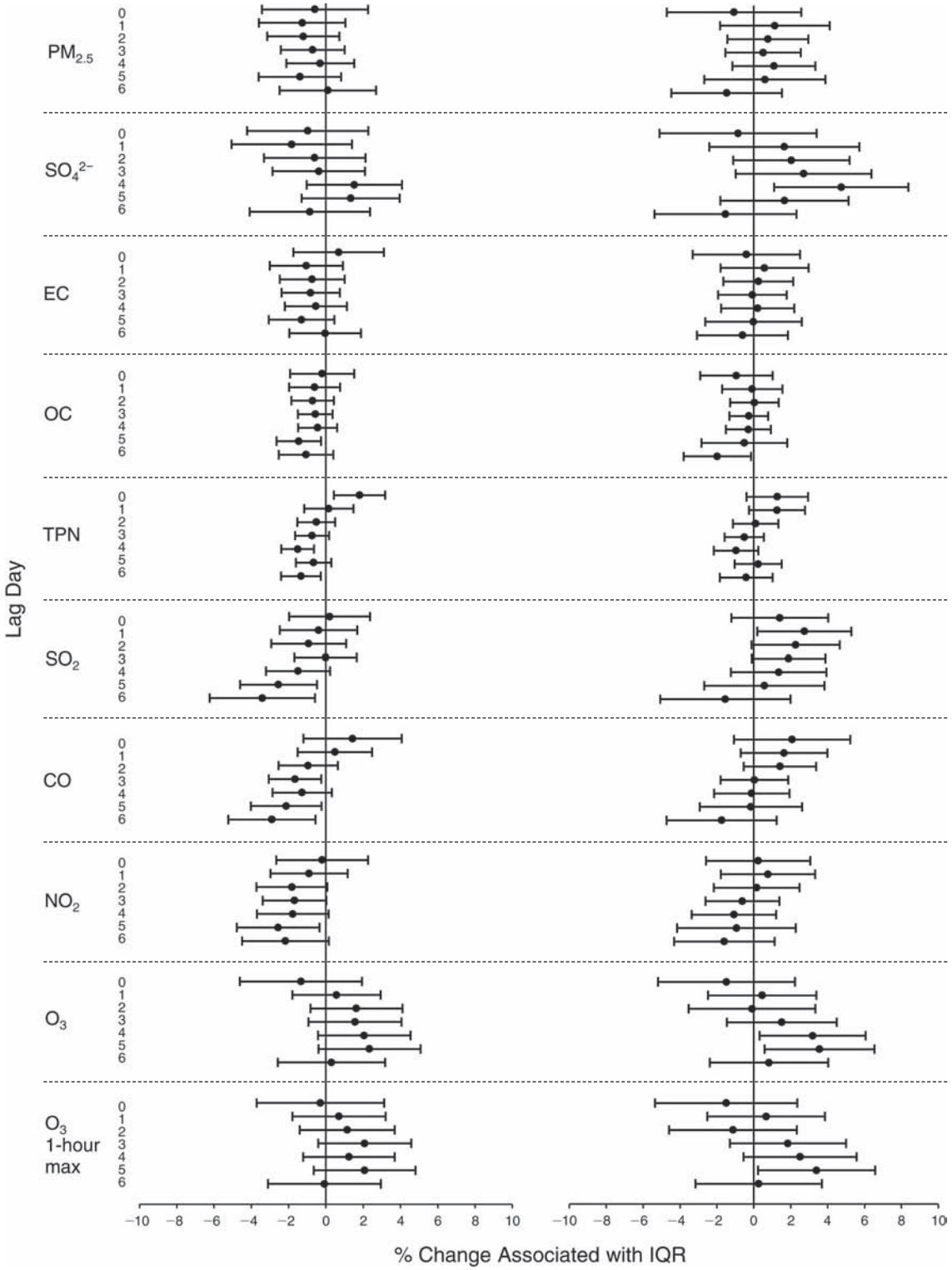


Figure L.24. Estimated means and 95% CIs for the percent change in WBC count associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 7-day moving average for temperature (df = 3), 6-day moving average for RH (df = 3), sex, and day of the week. Left: results including all observations; **Right:** results excluding observations on rainy days. Number of days observations were excluded: 4 out of 51 pre-Olympics; 5 out of 64 during Olympics; 3 out of 38 post-Olympics.

APPENDIX M. Percent Change in Biomarkers Associated with One IQR Increase in Pollutant Concentration, Controlling for Several Factors and Excluding Observations in the Post-Olympics Period

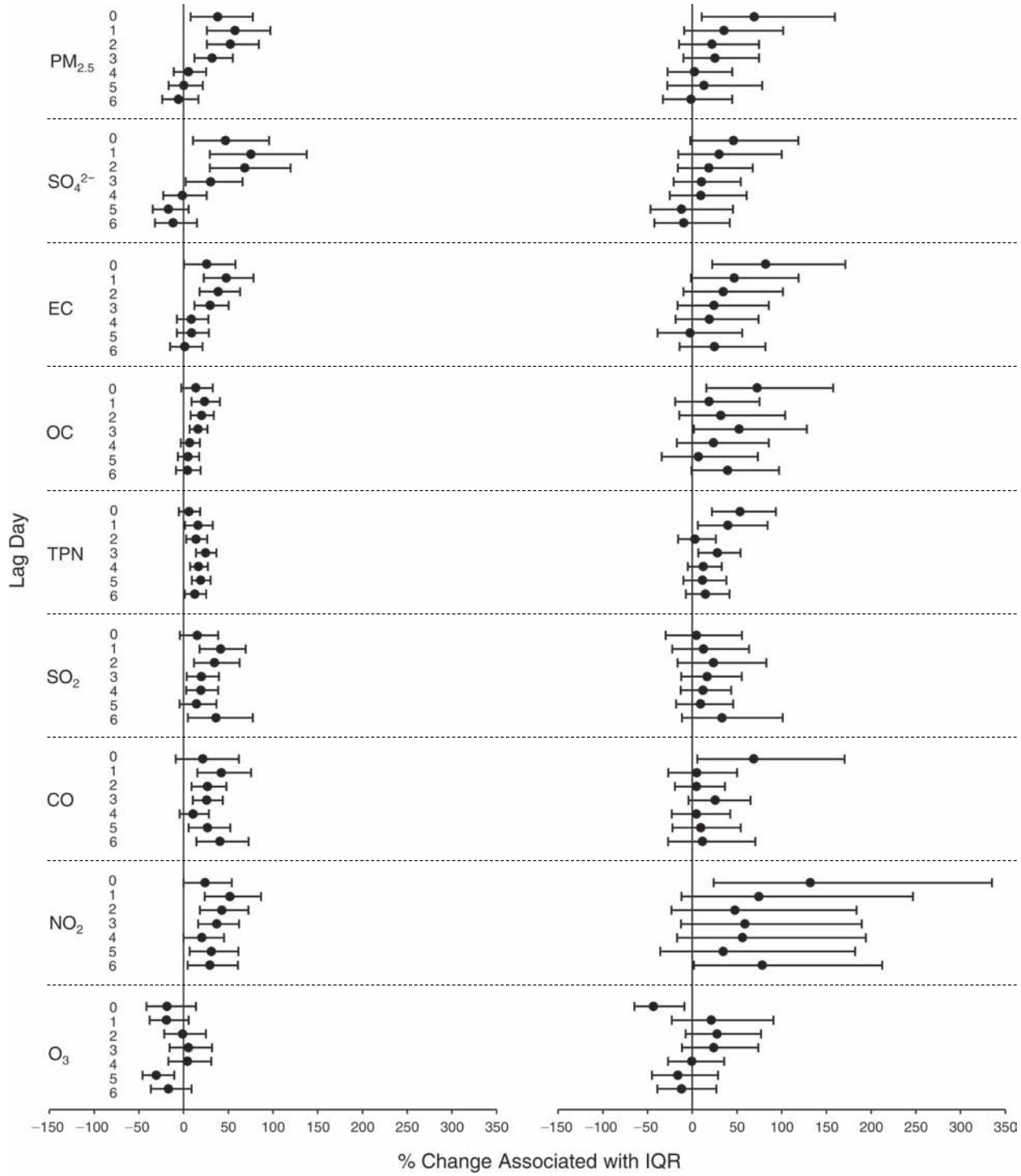


Figure M.1. Estimated means and 95% CIs for the percent change in urinary 8-OHdG (corrected by creatinine) associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 7-day moving average of temperature (df = 1), 2-day moving average of RH (df = 3), sex, and day of the week. Left: results including all three periods; **Right:** results of the pre- and during-Olympics periods only.

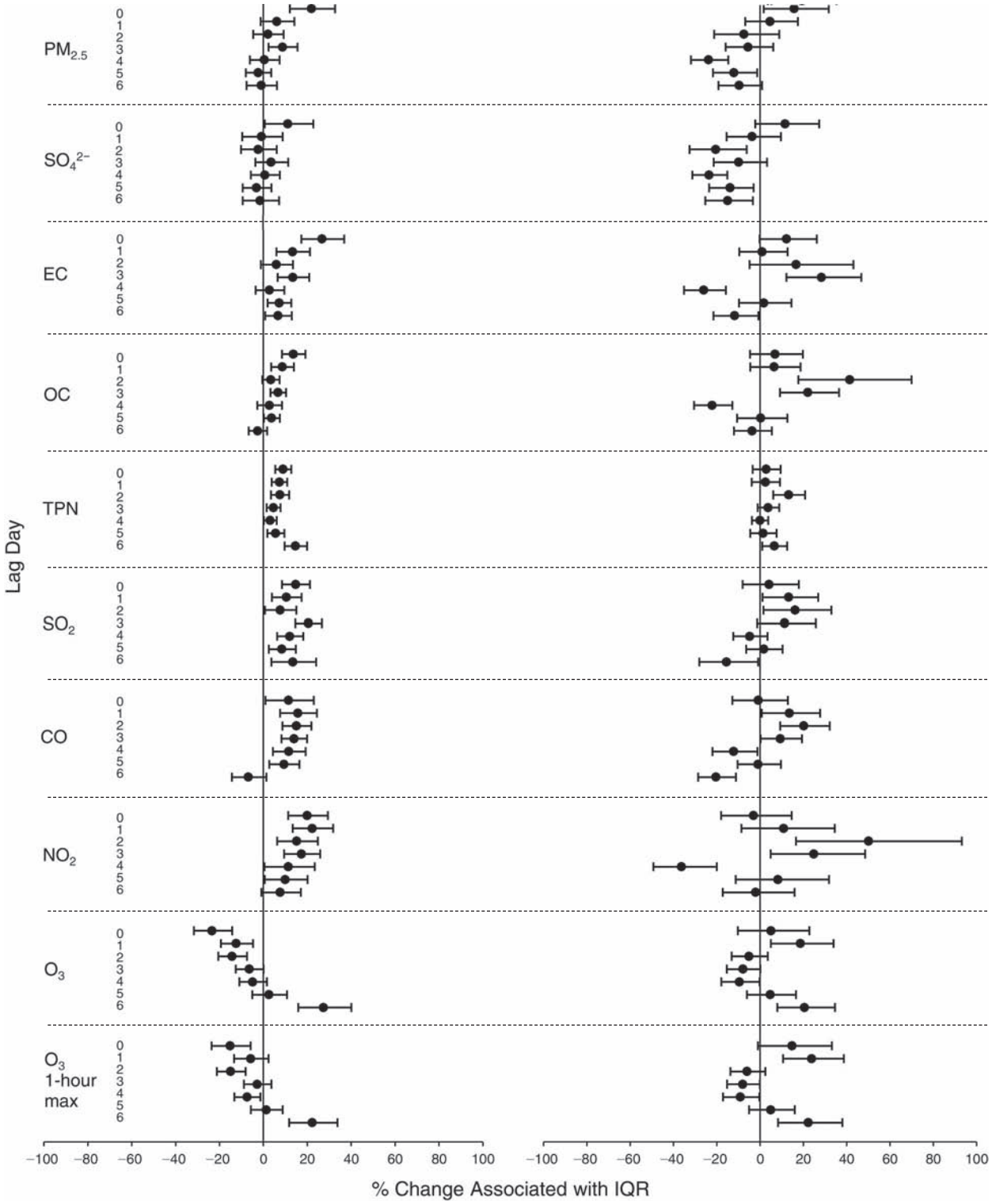


Figure M.2. Estimated means and 95% CIs for the percent change in EBC nitrite associated with one IQR increase in pollutant concentration, controlling for temperature (df = 2), RH (df = 1), 7-day moving average of temperature (df = 3), 3-day moving average of RH (df = 3), sex, and day of the week. Left: results including all three periods; **Right:** results of the pre- and during-Olympics periods only.

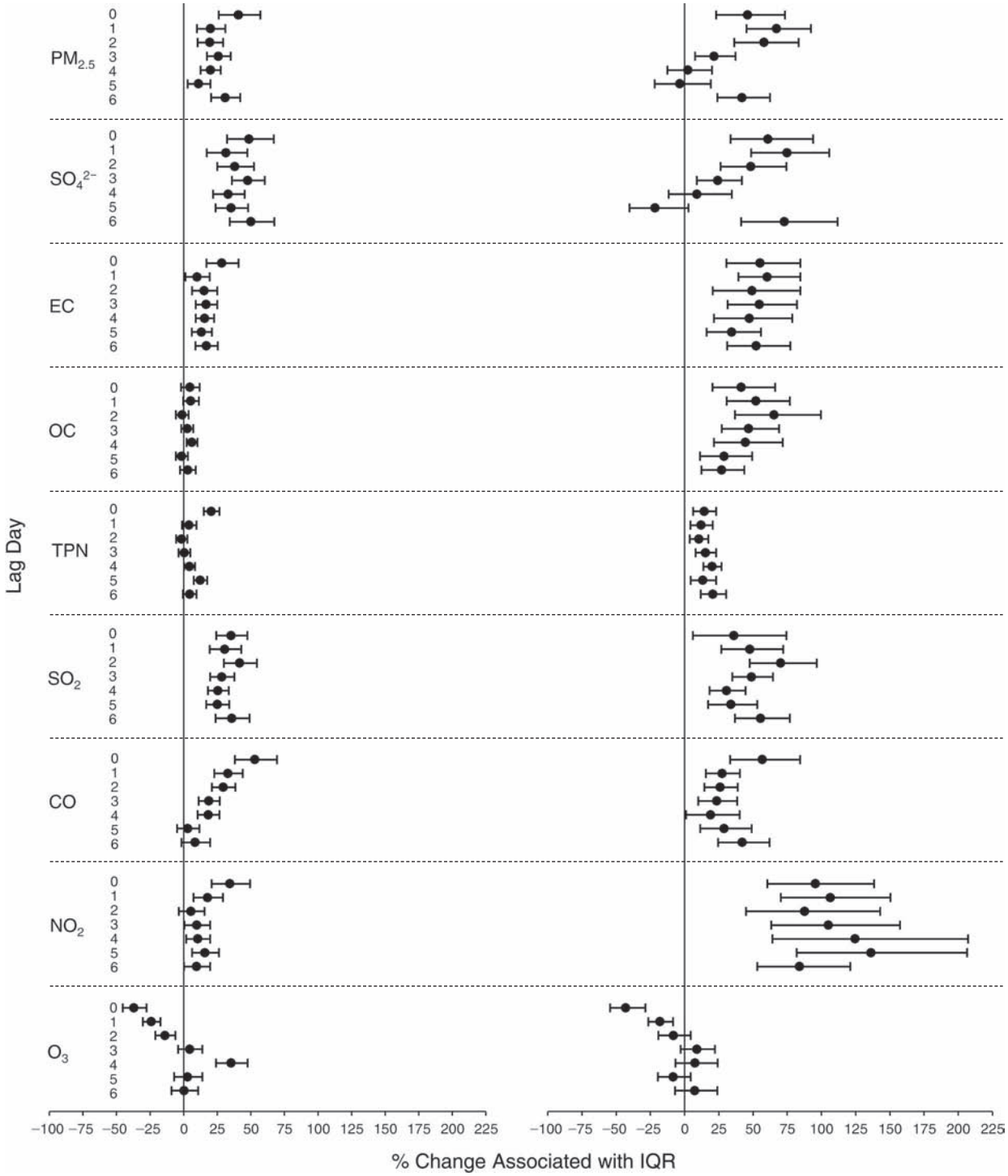


Figure M.3. Estimated means and 95% CIs for the percent change in FeNO associated with one IQR increase in pollutant concentration, controlling for temperature (df = 2), RH (df = 3), 7-day moving average of temperature (df = 2), 7-day moving average of RH (df = 3), sex, and day of the week. Left: results including all three periods; **Right:** results of the pre- and during-Olympics periods only.

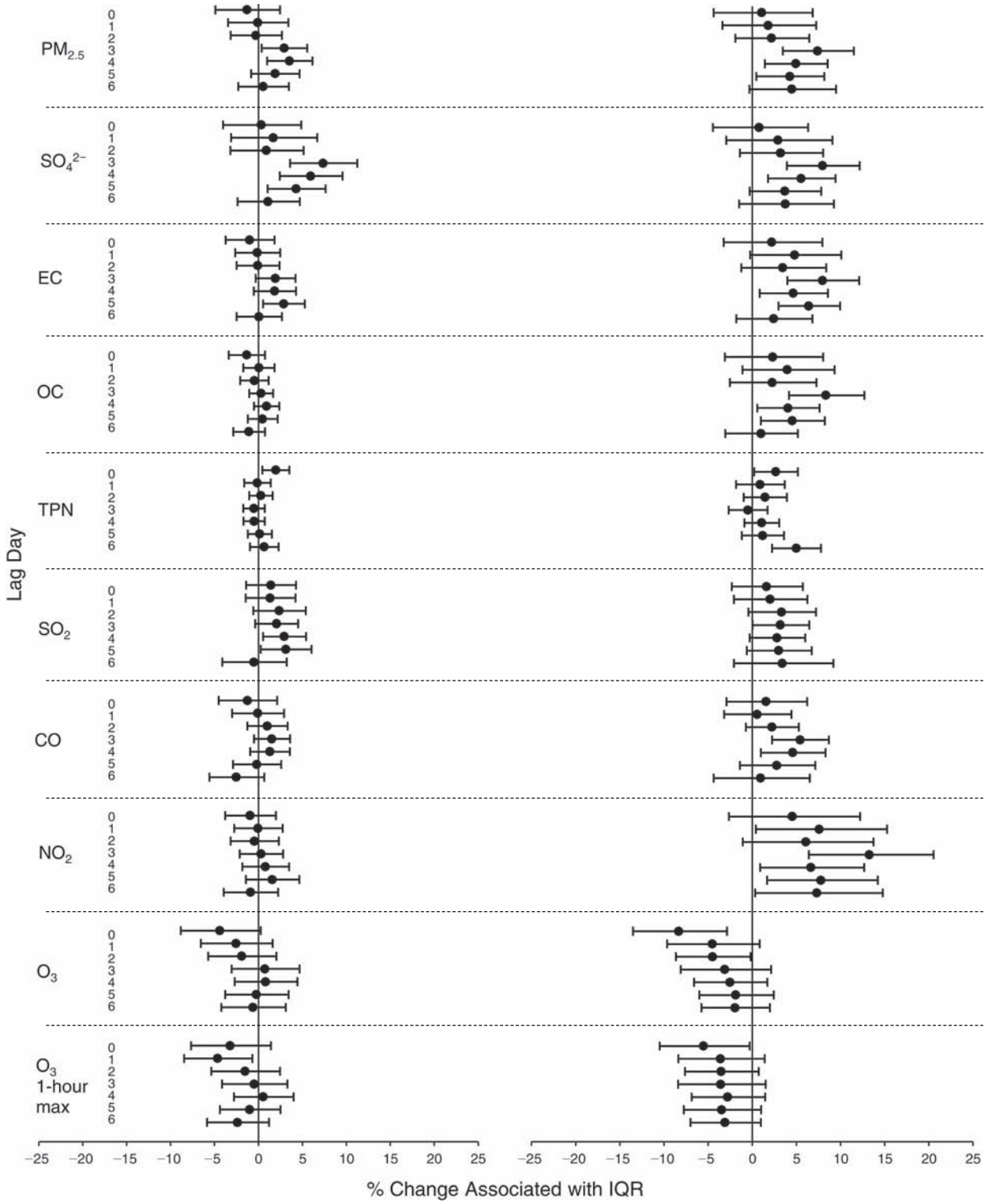


Figure M.4. Estimated means and 95% CIs for the percent change in sCD40L associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 1), 5-day moving average of temperature (df = 1), 2-day moving average of RH (df = 1), sex, and day of the week. Left: results including all three periods; **Right:** results of the pre- and during-Olympics periods only.

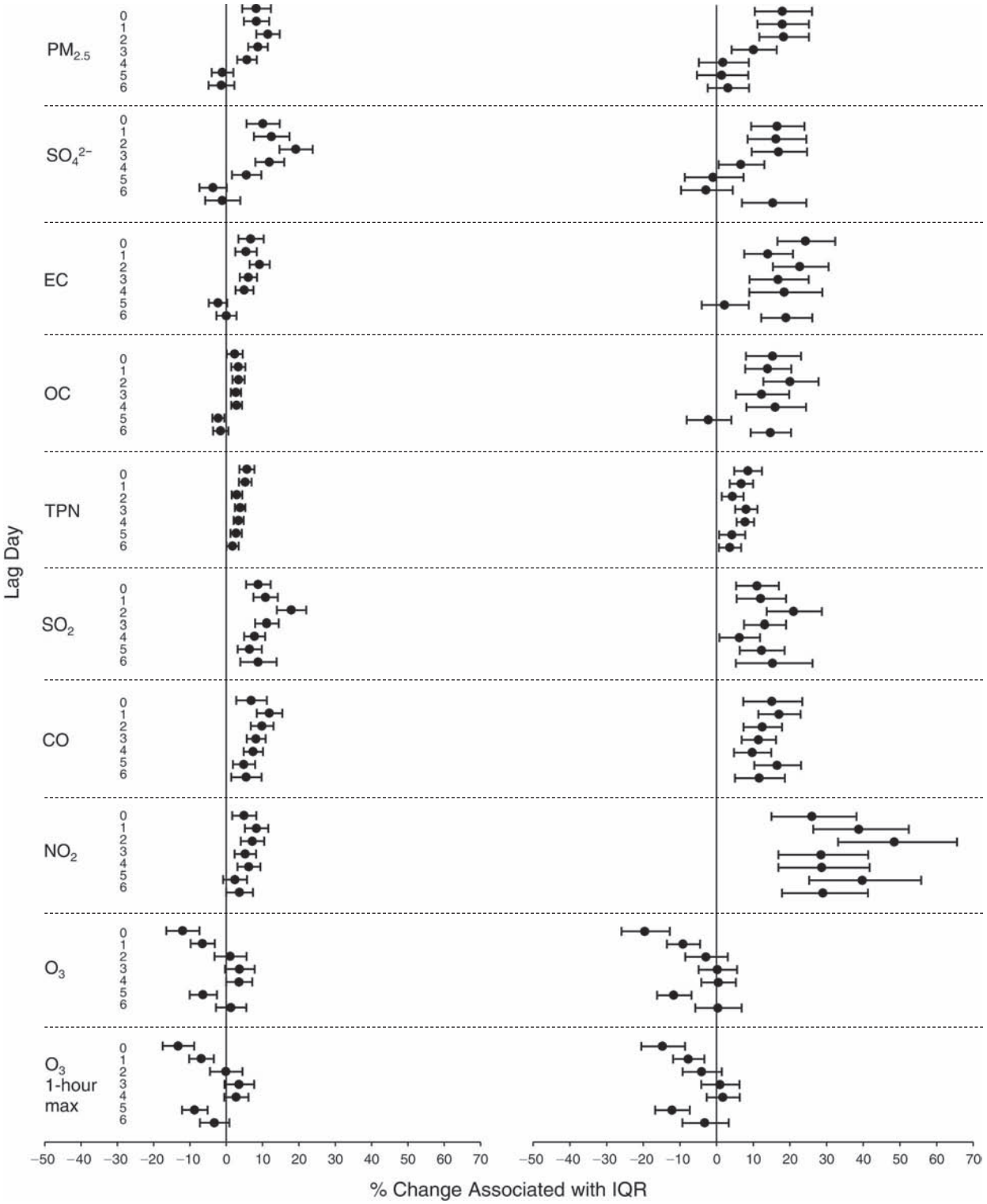


Figure M.5. Estimated means and 95% CIs for the percent change in sCD62P associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 3), 7-day moving average of temperature (df = 2), 4-day moving average of RH (df = 2), sex, and day of the week. Left: results including all three periods; **Right:** results of the pre- and during-Olympics periods only.

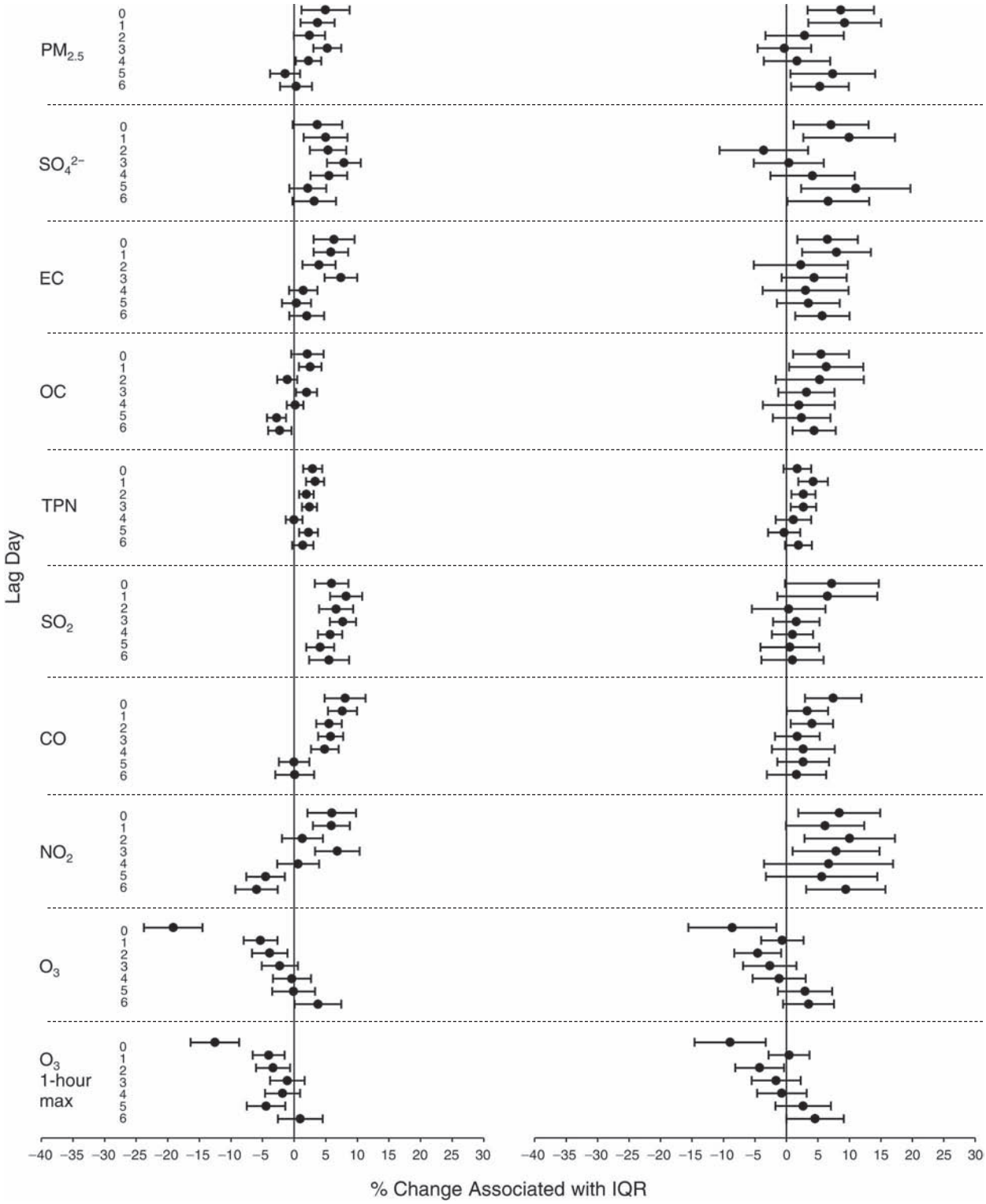


Figure M.6. Estimated means and 95% CIs for the percent change in vWF associated with one IQR increase in pollutant concentration, controlling for temperature (df = 3), RH (df = 3), 7-day moving average of temperature (df = 3), 6-day moving average of RH (df = 3), sex, and day of the week. Left: results including all three periods; **Right:** results of the pre- and during-Olympics periods only.

APPENDIX N. Percent Change in Biomarkers Associated with One IQR Increase in Pollutant Concentration, Controlling for Several Factors and With or Without Period Adjustment

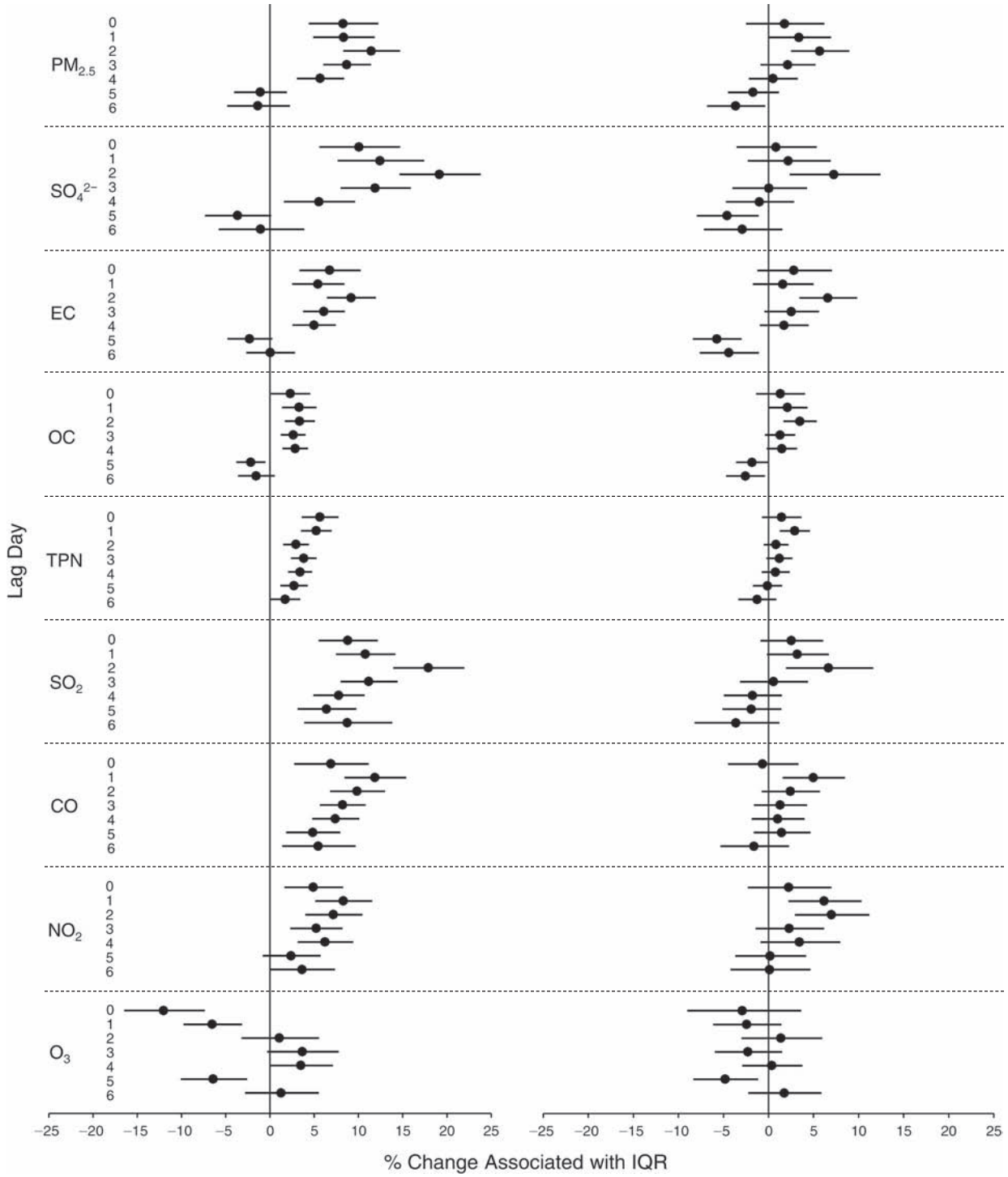


Figure N.1. Estimated means and 95% CIs for the percent change in sCD62P associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 3), 7-day moving average of temperature (df = 2), 4-day moving average of RH (df = 2), sex, and day of the week. Left: results for which the period factor was not adjusted; **Right:** results for which the period factor was adjusted.

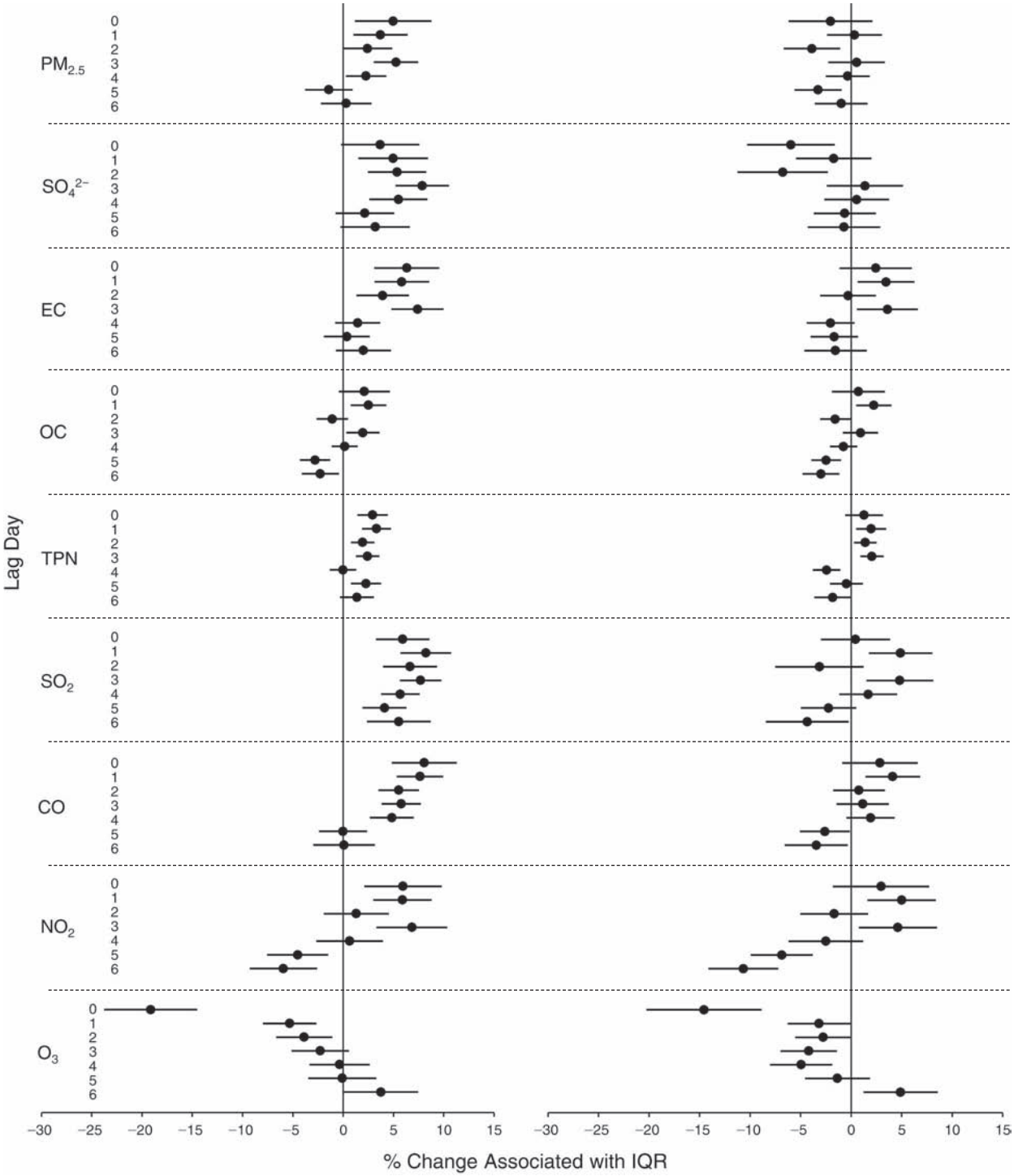


Figure N.2. Estimated means and 95% CIs for the percent change in vWF associated with one IQR increase in pollutant concentration, controlling for temperature (df = 3), RH (df = 3), 7-day moving average of temperature (df = 3), 6-day moving average of RH (df = 3), sex, and day of the week. Left: results for which the period factor was not adjusted; **Right:** results for which the period factor was adjusted.

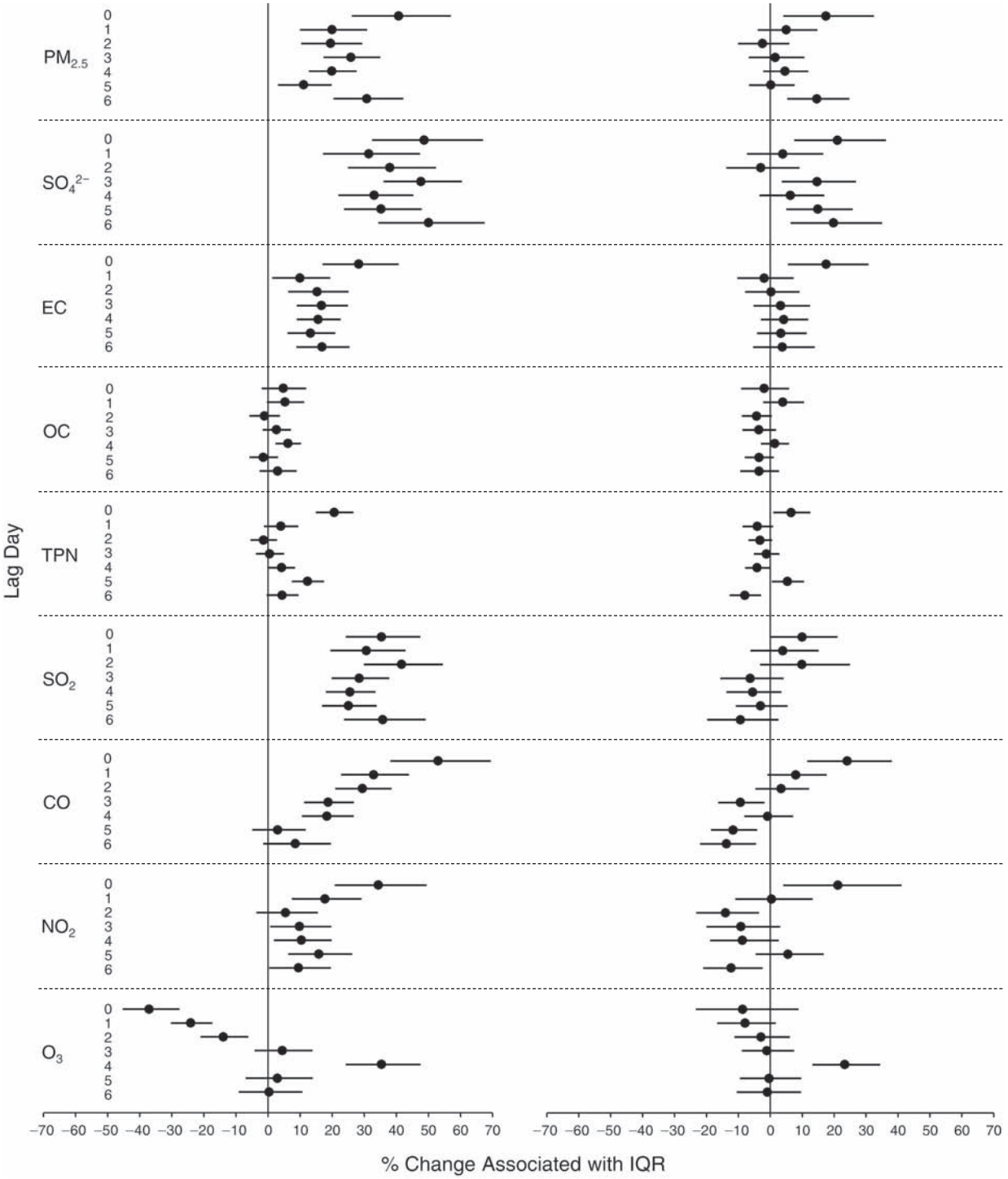


Figure N.3. Estimated means and 95% CIs for the percent change in FeNO associated with one IQR increase in pollutant concentration, controlling for temperature (df = 2), RH (df = 3), 7-day moving average of temperature (df = 2), 7-day moving average of RH (df = 3), sex, and day of the week. Left: results for which the period factor was not adjusted; **Right:** results for which the period factor was adjusted.

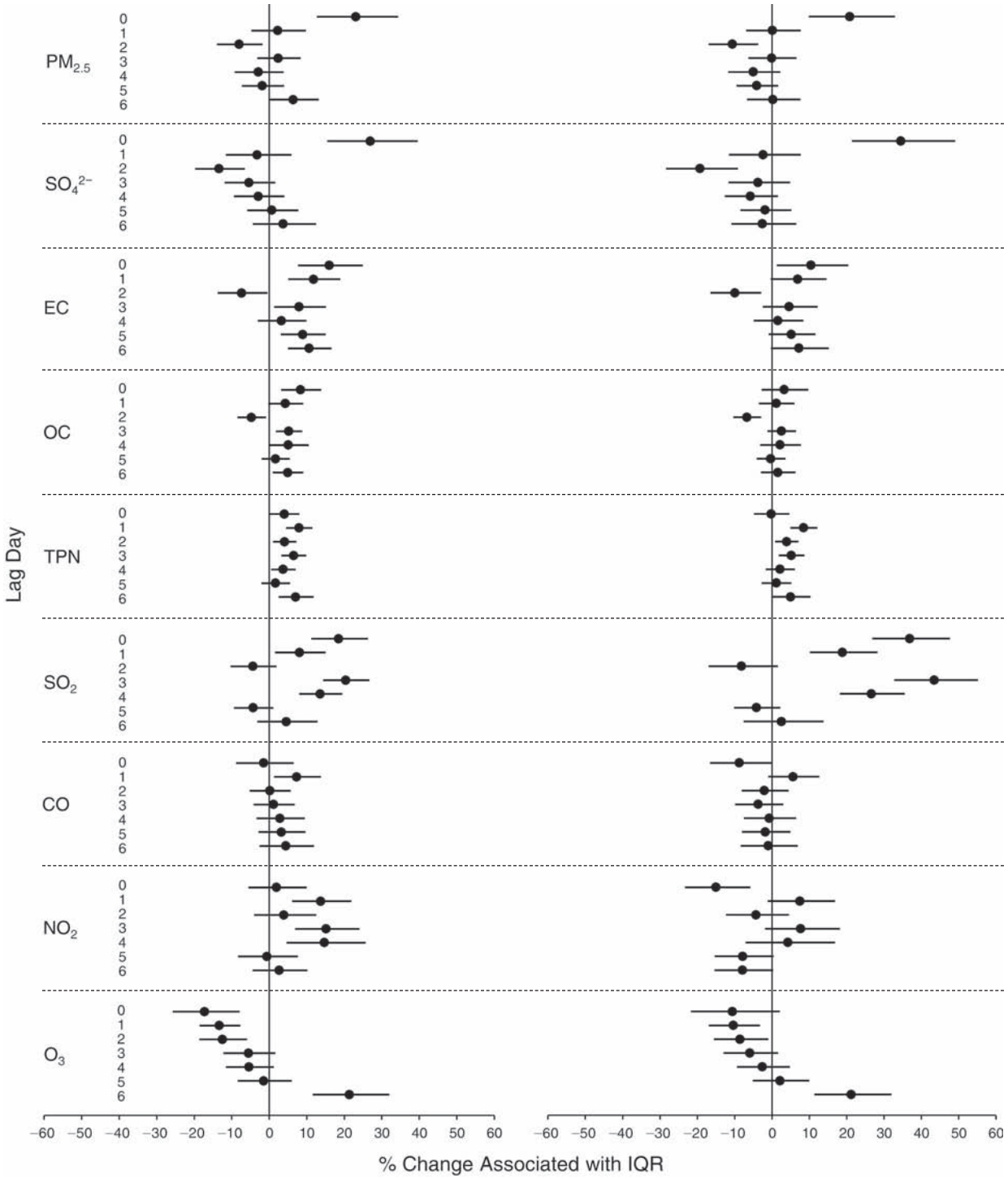


Figure N.4. Estimated means and 95% CIs for the percent change in EBC nitrite+nitrate associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 3), 7-day moving average of temperature (df = 3), and 5-day moving average of RH (df = 3), sex, and day of the week. Left: results for which the period factor was not adjusted; **Right:** results for which the period factor was adjusted.

APPENDIX O. Percent Change in Biomarkers Associated with One IQR Increase in Pollutant Concentration, Controlling for Several Factors, Including Subject as a Fixed or Random Effect

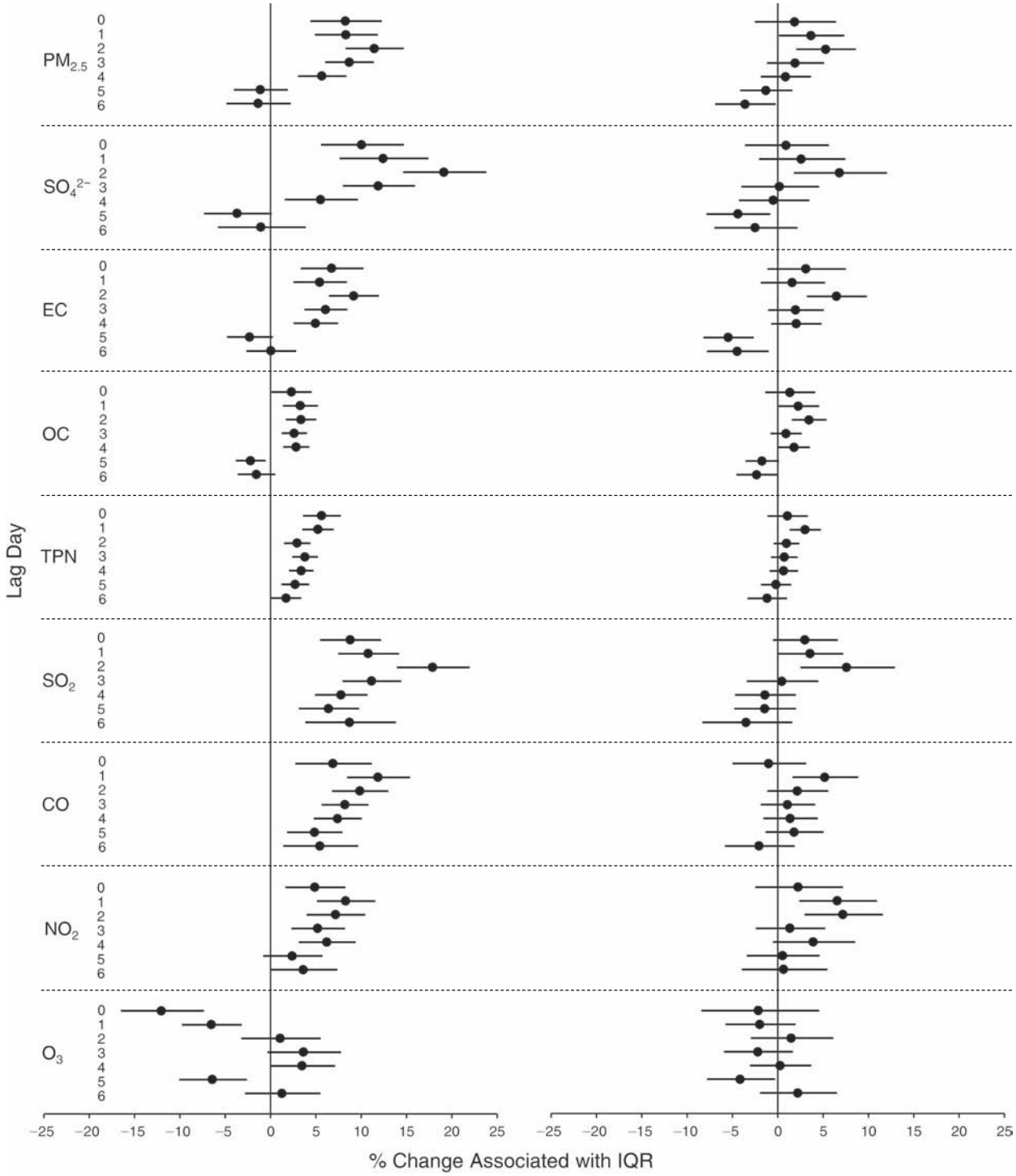


Figure O.1. Estimated means and 95% CIs for the percent change in sCD62P associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 3), 7-day moving average of temperature (df = 2), and 4-day moving average of RH (df = 2), sex, and day of the week. Left: results controlling for subject as a random effect; **Right:** results controlling for subject as a fixed effect.

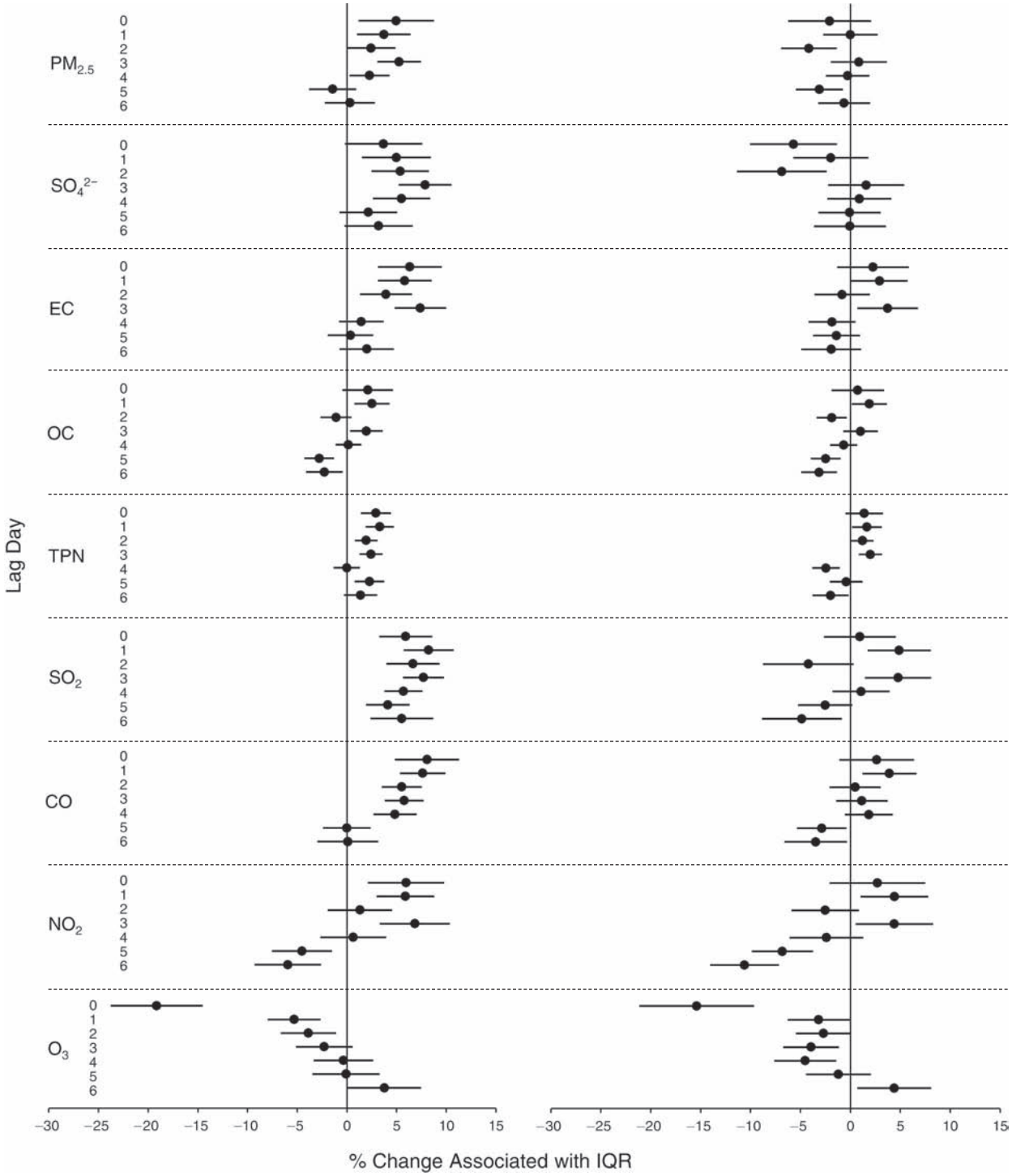


Figure O.2. Estimated means and 95% CIs for the percent change in vWF associated with one IQR increase in pollutant concentration, controlling for temperature (df = 3), RH (df = 3), 7-day moving average of temperature (df = 3), and 6-day moving average of RH (df = 3), sex, and day of the week, and treating the subject as a fixed effect. Left: results controlling for subject as a random effect; **Right:** results controlling for subject as a fixed effect.

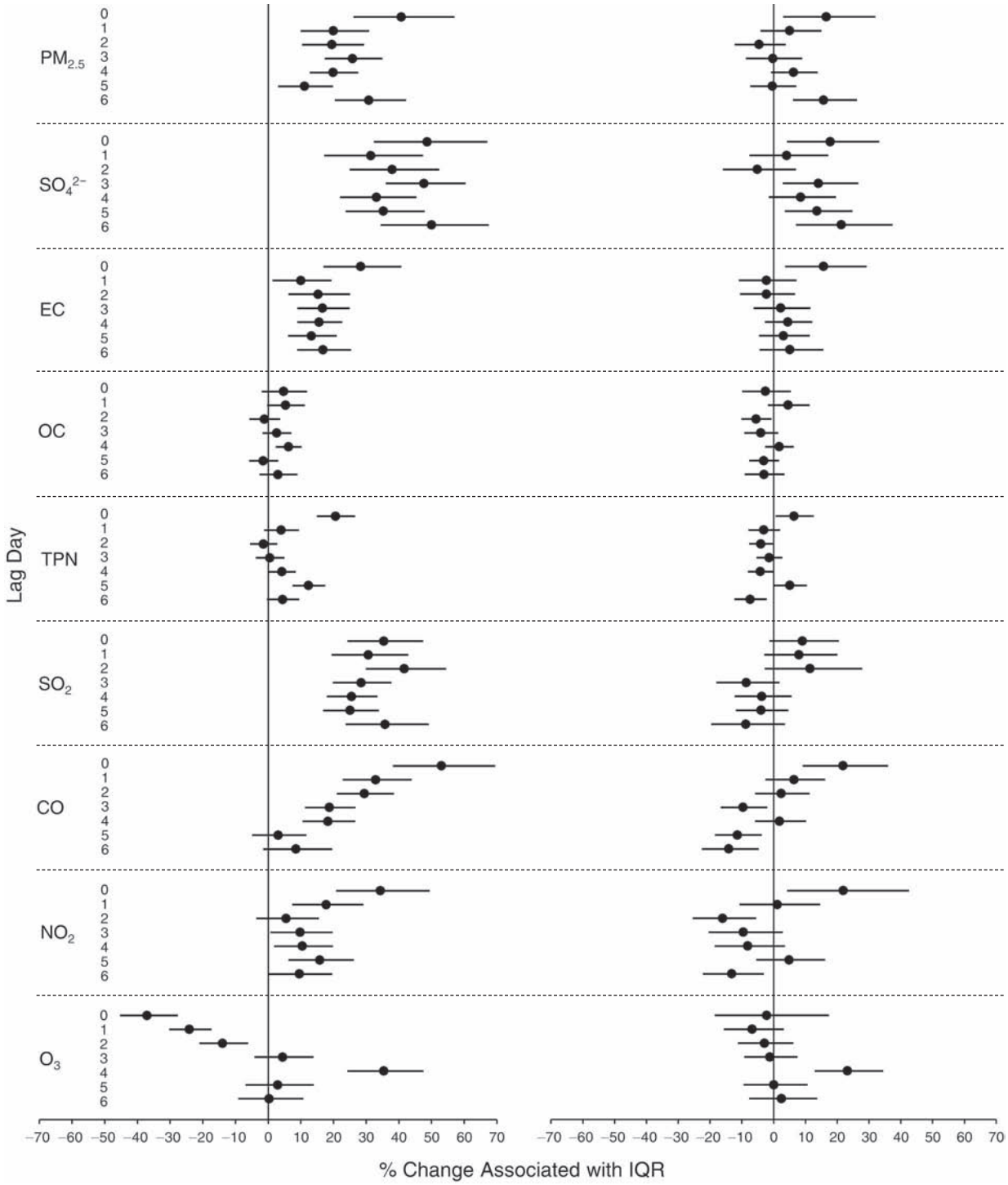


Figure O.3. Estimated means and 95% CIs for the percent change in FeNO associated with one IQR increase in pollutant concentration, controlling for temperature (df = 2), RH (df = 3), 7-day moving average of temperature (df = 2), and 7-day moving average of RH (df = 3), sex, and day of the week, and treating the subject as a fixed effect. Left: results controlling for subject as a random effect; **Right:** results controlling for subject as a fixed effect.

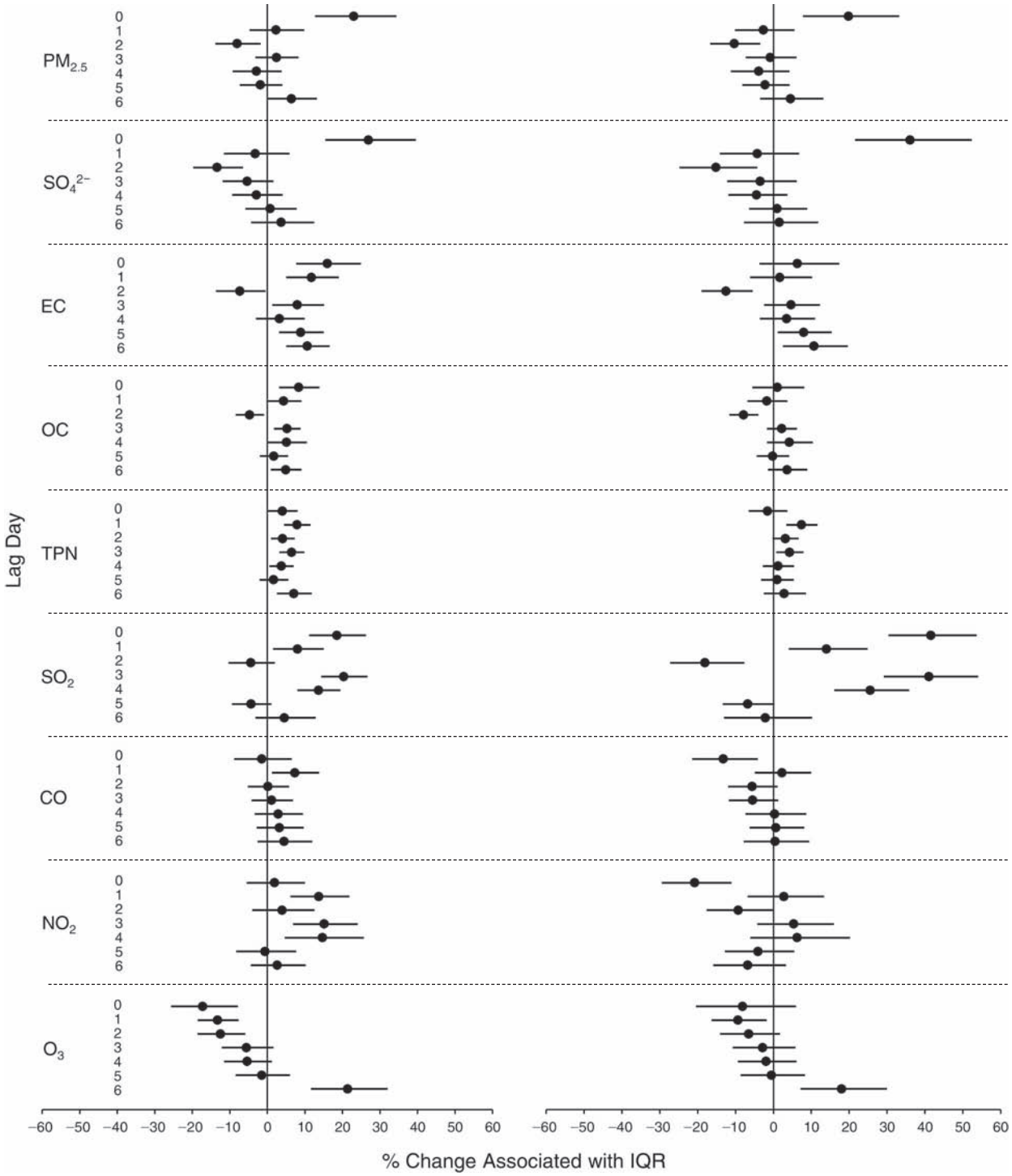


Figure O.4. Estimated means and 95% CIs for the percent change in EBC nitrite+nitrate associated with one IQR increase in pollutant concentration, controlling for temperature (df = 1), RH (df = 3), 7-day moving average of temperature (df = 3), and 5-day moving average of RH (df = 3), sex, and day of the week, and treating the subject as a fixed effect. Left: results controlling for subject as a random effect; **Right:** results controlling for subject as a fixed effect.

APPENDIX P. $PM_{2.5}$, Its Chemical Component Concentrations, and Particle Number, and Gaseous Pollutants by Period, with Mean and SD Based on Simple Algebraic Calculations

Table P.1. Statistics for $PM_{2.5}$ Mass and Chemical Components and Particle Number Concentration by Period Using Simple Algebraic Calculations[a]

Pollutant / Period	*n*	Mean	SD	Min	Median	Max	IQR
$PM_{2.5}$ (μg/m^3)							
Whole	100	85.2	51.9	14.6	80.6	268.2	76.8
Pre	35	100.9	38.8	24.4	95.8	219.1	39.3
During	33	69.4	42.9	14.6	59.2	171.4	66.5
Post	32	84.2	67.2	15.0	60.4	268.2	105.3
SO_4^{2-} (μg/m^3)							
Whole	92	21.8	17.0	1.0	20.7	73.9	28.0
Pre	35	28.4	12.8	5.4	29.6	65.0	16.9
During	28	23.2	19.4	2.0	20.7	73.9	32.0
Post	29	12.4	15.2	1.0	4.8	47.8	15.9
EC (μg/m^3)							
Whole	94	2.3	1.3	0.6	2.1	6.7	1.4
Pre	35	2.2	0.7	0.6	2.2	4.3	0.7
During	28	1.4	0.6	0.6	1.3	3.1	0.6
Post	31	3.3	1.6	1.0	3.3	6.7	2.3
OC (μg/m^3)							
Whole	94	10.2	6.6	1.1	8.2	43.3	5.1
Pre	35	8.8	3.3	1.1	8.2	21.9	2.2
During	28	6.9	2.7	2.7	6.5	14.0	2.8
Post	31	14.8	9.1	3.0	14.5	43.3	12.5
TPN (/cm^3)[b]							
Whole	95	15,950	4652	5189	15,710	29,395	6572
Pre	35	15,934	2856	9769	15,710	23,678	3171
During	30	12,595	3118	8065	12,115	19,316	3449
Post	30	19,324	5239	5189	20,053	29,395	6198

[a] All samples were above the detection limit. Summary statistics are based on 24-hr averages (from ~10 AM to ~10 AM next day).

[b] TPN indicates total particle number ranging from 13 nm to 764.7 nm.

Table P.2. Statistics for Gaseous Pollutants by Period Using Simple Algebraic Calculations[a]

Pollutant / Period	*n*	Mean	SD	Min	Median	Max	IQR
SO_2 (ppb)							
Whole	91	6.1	4.0	0.9	4.9	21.0	5.4
Pre	35	7.6	4.5	2.0	5.8	21.0	6.9
During	24	3.1	1.6	0.9	3.0	7.7	2.7
Post	32	6.6	3.6	0.9	6.2	14.9	5.0
CO (ppm)							
Whole	100	0.91	0.5	0.1	0.82	2.67	0.65
Pre	35	1.25	0.41	0.71	1.17	2.46	0.32
During	33	0.63	0.22	0.31	0.58	1.29	0.26
Post	32	0.82	0.59	0.1	0.74	2.67	0.85
NO_2 (ppb)							
Whole	100	27.0	15.3	9.5	24.7	80.7	18.7
Pre	35	26.0	5.1	15.8	25.3	41.2	5.4
During	33	13.9	4.6	9.8	13.0	30.0	2.5
Post	32	41.4	17.2	9.5	38.9	80.7	23.9
O_3 (ppb)							
Whole	100	29.1	17.0	3.5	25.1	69.1	25.4
Pre	35	31.8	16.4	5.3	34.1	64.3	23.5
During	33	39.5	16.0	10.0	38.4	69.1	17.3
Post	32	15.3	6.5	3.5	14.8	33.5	6.1
O_3 max (ppb)[b]							
Whole	100	63.3	30.9	12.4	63.6	132.7	47.6
Pre	35	65.8	31.7	12.7	65.9	123.5	45.7
During	33	80.4	28.1	26.2	82.3	132.7	39.3
Post	32	42.8	19.3	12.4	39.2	91.5	23.1

[a] All samples were above the detection limit. Summary statistics are based on 24-hr averages (from ~10 AM to ~10 AM next day).
[b] Maximum 1-hr average concentration within a 24-hr period.

APPENDIX Q. Period-Specific Means and SEs for Biomarker Measurements Based on Period Estimates from Mixed-Effects Models, Accounting for Repeated Measures But Not Adjusted for Covariates

Biomarker	Pre-Olympics Mean ± SE	During Olympics Mean ± SE	Post-Olympics Mean ± SE
Autonomic Dysfunction and Blood Pressure			
HR (bpm)[a]	66.6 ± 1.0	65.6 ± 1.0	65.7 ± 1.0
HF (ms^2)[a]	568.2 ± 1.1	558.0 ± 1.1	628.6 ± 1.1
LF (ms^2)[a]	467.3 ± 1.1	403.4 ± 1.1	401.7 ± 1.1
LF/HF[a]	0.83 ± 1.1	0.71 ± 1.1	0.64 ± 1.1
rMSSD (ms)[a]	54 ± 1.1	54 ± 1.1	52 ± 1.1
SDNN (ms)[a]	60 ± 1.0	58 ± 1.0	59 ± 1.0
VLF (ms^2)[a]	648.4 ± 1.1	597.1 ± 1.1	651.5 ± 1.1
Total power (ms^2)[a]	1958.1 ± 1.1	1825.2 ± 1.1	1974.3 ± 1.1
DBP (mmHg)	60.5 ± 0.6	60.0 ± 0.6	61.5 ± 0.6
SBP (mmHg)	104.6 ± 0.9	103.3 ± 0.9	106.6 ± 0.9
Systemic Inflammation and Oxidative Stress			
CRP (% ≥ 0.3 mg/L)	55	46	36
Fibrinogen (g/L)	2.46 ± 0.03	2.41 ± 0.03	2.83 ± 0.03
RBCs ($\times 10^{12}$/L)	4.59 ± 0.04	4.60 ± 0.04	4.47 ± 0.04
WBCs ($\times 10^9$/L)	5.24 ± 0.10	5.31 ± 0.10	5.10 ± 0.10
Lymphocytes ($\times 10^9$/L)	1.66 ± 0.03	1.71 ± 0.03	1.56 ± 0.03
Neutrophils ($\times 10^9$/L)	3.06 ± 0.08	3.09 ± 0.08	3.11 ± 0.08
Urinary 8-OHdG (mg/mol creatinine)[a]	3.70 ± 1.12	2.22 ± 1.12	3.34 ± 1.12
Pulmonary Oxidative Stress and Inflammation			
FeNO (ppb)[a]	11.76 ± 1.04	5.80 ± 1.04	12.51 ± 1.04
EBC			
Nitrite (μM)[a]	7.33 ± 1.03	4.71 ± 1.03	4.69 ± 1.04
Nitrate (μM)[a]	2.79 ± 1.04	2.61 ± 1.04	4.23 ± 1.05
Nitrite+nitrate (μM)[a]	10.48 ± 1.03	7.68 ± 1.03	10.37 ± 1.03
pH	7.43 ± 0.03	7.46 ± 0.03	7.61 ± 0.03
8-Isoprostane (% ≥ 1.56 pg/ml)	68	44	74
Hemostasis			
sCD62P (ng/mL)[a]	6.49 ± 1.02	5.03 ± 1.02	5.34 ± 1.02
sCD40L (ng/mL)[a]	1.89 ± 1.02	1.77 ± 1.02	1.90 ± 1.02
Platelet aggregation (%)	58.47 ± 1.55	63.33 ± 1.55	57.78 ± 1.55
vWF (%)	102.1 ± 2.5	90.0 ± 2.5	83.8 ± 2.5

[a] Biomarker had skewed data distributions, so geometric means are shown.

APPENDIX R. HEI Quality Assurance Statement

The conduct of this study was subjected to independent audits by David Bush of T&B Systems, Inc. Bush is an expert in quality assurance for air quality monitoring studies and data management. The audits included on-site reviews of study activities for conformance to the study protocol and operating procedures, and selected performance audits of monitoring equipment. The dates of the audits are listed here with the phase of the study examined.

QUALITY ASSURANCE AUDITS

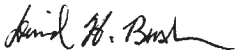
October 6–8, 2008

The auditors conducted on-site audits at the Peking University First Hospital and Peking University, in Beijing, China, during the subject recruitment and testing period. Ellen Miles, an independent consultant, participated in this audit, providing expertise for the review of the clinical portions of the study. No significant issues were noted, though several recommendations were presented for improving documentation of SOPs and QC activities.

June 14, 2012

The auditor reviewed the study final report, as well as the final data set used in the analysis, during an on-site visit to the Keck School of Medicine, University of Southern California. Several data points for each measurement were traced through the entire data management sequence to verify the integrity of the data set. No significant issues were noted.

Written reports of each inspection were provided to the HEI project manager, who transmitted the findings to the principal investigator. These quality assurance audits demonstrated that the study was conducted by an experienced team with a high concern for data quality. The report appears to be an accurate representation of the study.



David H. Bush, Quality Assurance Officer

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OTHER PUBLICATIONS RESULTING FROM THIS RESEARCH

Gong J, Zhu T, Kipen H, Wang G, Hu M, Ohman-Strickland P, Lu S-E, Zhang L, Wang Y, Zhu P, Rich DQ, Diehl SR, Huang W, Zhang J. 2013. Malondialdehyde in exhaled breath condensate and urine as a biomarker of air pollution induced oxidative stress. J Expo Sci Environ Epidemiol (doi: 10.1038/jes.2012.127) [E-pub ahead of print.]

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ABBREVIATIONS AND OTHER TERMS

8-OHdG	8-hydroxy-2′-deoxyguanosine
ACF	autocorrelation function
ADR	adrenaline
AIC	Akaike Information Criterion
ATS/ERS	American Thoracic Society/European Respiratory Society
BP	blood pressure
CRP	C-reactive protein
DBP	diastolic blood pressure
EBC	exhaled breath condensate
EC	elemental carbon
ECD	electrochemical detection
ECG	electrocardiography
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
eNO	exhaled nitric oxide
FeNO	fractional exhaled nitric oxide
GC–MS	gas chromatography–mass spectroscopy
HEART	Health Effects of an Air Pollution Reduction Trial
HF	high frequency power (0.15–0.40Hz)
HPLC	high performance liquid chromatography
HR	heart rate
HRP	horseradish peroxidase
HRV	heart rate variability
IC	ion chromatography
ICP–MS	inductively coupled plasma mass spectrometer
IQR	inter-quartile range
IRB	institutional review board
LF	low frequency power (0.04–0.15 Hz)
OC	organic carbon
OM	organic matter
PAH	polycyclic aromatic hydrocarbon
PM	particulate matter
PM_1	particulate matter ≤ 1 μm in aerodynamic diameter, or ultrafine particles
$PM_{2.5}$	PM ≤ 2.5 μm in aerodynamic diameter
PM_{10}	particulate matter ≤ 10 μm in aerodynamic diameter
PPP	platelet-poor plasma
PRP	platelet-rich plasma
RBC	red blood cell
RFPA	request for preliminary applications
RH	relative humidity

rMSSD	root mean square of successive differences between adjacent normal cycles (successive NN intervals)
ROS	reactive oxygen species
SBP	systolic blood pressure
sCD40L	soluble CD40 ligand
sCD62P	P-selectin
SDNN	standard deviation of normal to normal (R–R intervals)
SMPS	scanning mobility particle sizer
TDMPS	twin differential mobility particle sizer
TEOM	tapered element oscillating microbalance
TMB	tetramethylbenzidine
TPN	total particle number
UMDNJ	University of Medicine and Dentistry of New Jersey
U.S. EPA	U.S. Environmental Protection Agency
VLF	very low frequency power (0.003–0.04 Hz)
vWF	von Willebrand factor
WBC	white blood cell

COMPOUNDS, IONS, AND ELEMENTS

Al	aluminum
As	arsenic
Ba	barium
Ca/Ca^{2+}	calcium/calcium ion
Cd	cadmium
Cl^-	chloride
CO	carbon monoxide
Co	cobalt
Cr	chromium
Cu	copper
F^-	fluoride
Fe	iron
H_2SO_4	sulfuric acid
K/K^+	potassium/potassium ion
Mg/Mg^{2+}	magnesium/magnesium ion
Mn	manganese
Mo	molybdenum
Na/Na^+	sodium/sodium ion
NH_4^+	ammonium
Ni	nickel
NO	nitric oxide
NO_2	nitrogen dioxide
NO_x	nitrogen oxides
O_3	ozone
O_3 max	maximum 1-hour average O_3 concentration within a 24-hour period
P	phosphorus
Pb	lead
Se	selenium
SO_2	sulfur dioxide
SO_4^{2-}	sulfate
Th	thorium
Ti	titanium
Tl	thallium
TPN	total particle number
U	uranium
V	vanadium
Zn	zinc

Research Report 174, *Cardiorespiratory Biomarker Responses in Healthy Young Adults to Drastic Air Quality Changes Surrounding the 2008 Beijing Olympics*, J. Zhang et al.

INTRODUCTION

As the accompanying Preface describes, over the last several years HEI's Outcomes Research Program has been designed to evaluate the effects of regulatory and other actions taken to improve air quality. The overall goal has been to provide evidence about the extent to which air quality regulations or other actions may or may not have improved air quality and health. Some of these HEI-funded studies (e.g., Peel et al. 2010) looked at the effects of interventions lasting only a limited period of time; these studies did not assess the effect of a longer-term regulatory act, but rather took advantage of a unique event (such as the 1996 Atlanta Olympics, in the case of Peel and colleagues) during which traffic or other changes were to be made locally with the goal of improving air quality for the duration of the event. The current study took advantage of a similar unique event.

HEI periodically issues a request for preliminary applications (RFPA) for novel research on the health effects of air pollutants derived from motor vehicle emissions. In 2006, in response to RFPA 05-3, "Health Effects of Air Pollution," Dr. Junfeng (Jim) Zhang, then of the University of Medicine and Dentistry of New Jersey–School of Public Health and the Environmental and Occupational Health Sciences Institute at Rutgers University, submitted a preliminary application "Health Impact of Changes in Air Pollution Levels and PM Composition Brought by the 2008 Olympic Games in Beijing." The goal of the study was to measure levels of air pollutants and to evaluate prospectively the impact on multiple cardiopulmonary responses of changes in air pollution levels and composition following interventions before, during, and after the 2008 Beijing Olympic Games. The investigator indicated that the Chinese government was launching "a series of aggressive policies to reduce local and regional emissions that affect air quality in the greater Beijing metropolitan area. The implementation of these policies is expected to result in a gradual decline of PM (particulate matter) and other air pollutants over the years to come. In addition, there will be an expected sudden and more dramatic decline in air pollution levels during the Games due to more aggressive but temporary control of air pollution (e.g., traffic restraints and redirections, domestic and industrial emission restraints)."

HEI's Research Committee thought the intervention provided a unique opportunity to assess the health effects of likely reductions in pollutant emissions and asked Dr. Zhang to submit a full proposal on this topic. The investigator was asked to describe in detail the interventions that would take place, as well as provide an estimate of the expected reductions in air pollutant levels. In response, Zhang submitted a proposal that focused on the evaluation of acute cardiopulmonary responses in healthy volunteers — primarily medical residents who worked and resided on the campus of the First Hospital of Peking University, located in the center of Beijing. In collaboration with colleagues in China and the United States, Zhang's team would measure markers of cardiovascular and pulmonary function, respiratory and systemic inflammation, and oxidative stress. Pollutant levels and health endpoints would be measured in three periods, each approximately 4 to 7 weeks, immediately before, during, and immediately after the Olympics. Zhang and colleagues would determine whether changes in cardiopulmonary responses could be associated with changes in PM concentrations and composition. After discussions with the Research Committee, Zhang submitted a revised proposal that the Committee recommended for funding.

This Commentary is intended to aid the sponsors of HEI and the public by highlighting both the strengths and limitations of the study and by placing the Investigators' Report into scientific and regulatory perspective.

Dr. Zhang's 3-year study, "Molecular and Physiological Responses to Drastic Changes in PM Concentration and Composition," began in July 2007. Total expenditures were $562,803. Zhang and colleagues submitted their report for review in May 2011. A revised report, received in February 2012, was accepted for publication in the same month. During the review process, the HEI Health Review Committee and the investigators had the opportunity to exchange comments and to clarify issues in both the Investigators' Report and the Review Committee's Commentary.

* A list of abbreviations and other terms appears at the end of the Investigators' Report.

SCIENTIFIC BACKGROUND

$PM_{2.5}$ AND CARDIOPULMONARY EFFECTS

At the time the study started, epidemiologic and controlled exposure studies indicated that acute exposure to $PM_{2.5}$ (particulate matter ≤ 2.5 µm in aerodynamic diameter) affected cardiopulmonary morbidity and mortality; however, the pathways by which PM exposure affected those endpoints were not well characterized. Several plausible and not mutually exclusive mechanistic pathways were suggested (reviewed in Brook et al. 2004). These involved changes in autonomic control (heart rate and heart rate variability); coagulation and thrombosis pathways (including platelet aggregation, levels of fibrinogen, von Willebrand factor [vWF], soluble P-selectin [sCD62P], soluble CD40 ligand [sCD40L]); and oxidant and inflammatory mechanisms (including formation of reactive oxygen species and increases in markers of airway and systemic inflammation [cytokines, C-reactive protein, inflammatory cells, and markers in exhaled breath]). Brook and colleagues subsequently updated their review (2010), indicating that the evidence to causally link exposure to PM and cardiovascular morbidity and mortality was stronger. However, the mechanistic pathways by which PM might affect these health endpoints are still not firmly established.

In the current study, Zhang and colleagues took advantage of a unique situation — anticipated changes in levels of air pollution — to evaluate the acute impact of changes in exposure to air pollutants on multiple biomarkers of cardiovascular response. The aim was both to elucidate the pathways involved in acute responses to air pollutants and to provide additional evidence for acute responses after exposure to air pollution.

AIR POLLUTION CONTROL MEASURES AROUND THE 2008 OLYMPIC GAMES

The substantial actions taken by the Chinese government in and around Beijing during the 2008 Olympic and Paralympic Games to reduce air pollution had the goal of limiting both vehicular traffic from Beijing roads and the emissions from industrial, power generation, and commercial facilities in Beijing, as well as construction activities. In his application to HEI, Dr. Zhang indicated that the interventions aimed to reduce pollutant levels from the 2004 annual means of 195 µg/m^3 for PM_{10} (particulate matter ≤ 10 µm in aerodynamic diameter) and 105 µg/m^3 for $PM_{2.5}$ to around 50 and 30 µg/m^3, respectively, during the Olympics.

Figure 2* of the Investigators' Report indicates that "full-scale control" measures were in place August 8–23, 2008 (the Olympic Games), and September 7–19, 2008 (the Paralympic Games), in Beijing. Full-scale control measures included an odd/even number plate rule for traffic control, reductions or cessations in the operations of factories in the Beijing area, the stopping of outdoor construction activities, temporary closings of some gas stations, and increases in bus fleet and transit frequency. Figure 2 of the Investigator's Report also indicates that some control measures were put in place before July 20, 2008, and the implementation of full-scale controls. These additional pre-Olympics measures included the introduction of new vehicular emissions standards, the relocation of heavy industrial polluters, and further restrictions on vehicles allowed to drive into Beijing.

PRIOR INTERVENTION STUDIES

As described in the accompanying Preface and in a review by van Erp and colleagues (2012), other intervention studies have taken advantage of either "natural" experiments or planned changes in regulations. Two types of such interventions are particularly relevant to the current study: traffic restrictions and factory closings.

Traffic-Restriction Interventions

Although there has been substantial progress in reducing emissions from the transportation sector, these emissions remain a major contributor to urban air pollution. With a large segment of the population living in close proximity to traffic sources, exposure to traffic-related air pollutants is an important public health concern. HEI's recent review of this topic (HEI Panel on the Health Effects of Traffic-Related Air Pollution 2010) concluded that there is sufficient evidence to support a causal association between exposure to traffic-related pollution and asthma exacerbation; studies that have evaluated the relationship between the distance from residences or schools to busy roads and health outcomes (e.g., respiratory symptoms, asthma incidence, pulmonary function, or mortality) suggest a causal relationship.

Because of the presumed public health impacts of reductions in exposure to traffic-related air pollution, many countries have implemented regulations to reduce such exposures. Examples are regulations aimed at reducing sulfur in fuel, mandating "low emission zones" — that is, restricting older vehicles with relatively high emission

*Figure 2 is based on information from a study by M. Wang and colleagues (2009), which was in turn based on information available from a Chinese government Web site.

levels from entering downtown areas, as is being done in an increasing number of cities in Europe and elsewhere — or targeting traffic congestion. Measures to reduce congestion in major urban areas include charging a fee for vehicles to enter the area (e.g., in London [see Kelly et al. 2011], Milan, Singapore, and Stockholm [see Hugosson et al. 2006]), banning entry of nonresidents' vehicles (in Rome [see Cesaroni et al. 2012]), and imposing restrictions on when residents may use their vehicles (in Mexico City, Athens, and Budapest). A few studies have evaluated the effects of these longer-term traffic management schemes on air pollution levels: Kelly and colleagues (2011) were unable to detect a clear effect on air quality of the introduction of London's Congestion Charging Scheme. This was most likely attributable to a variety of factors, including meteorologic conditions and concurrent changes in other regulatory programs that may have increased levels of some pollutants. Other (modeling) studies have shown relatively small beneficial effects on air pollution levels of long-term traffic management schemes such as low emission zones (Johansson et al. 2009; Cesaroni et al. 2012).

In addition to the current study and others that have evaluated the effects of pollutant changes around the Beijing Olympic Games (discussed in detail below in the "Health Review Committee's Evaluation of the Study" section), a few other studies have evaluated the impact of short-term reversible traffic management schemes on measured air pollution levels and health. Studies by Friedman and colleagues (2001) and Peel and colleagues (2010) assessed the effects of a temporary reduction in traffic congestion at a prior Olympic Games in Atlanta in 1996. Friedman's team reported that the traffic intervention resulted in a decrease in acute care visits for pediatric asthma and a concomitant decrease in concentrations of ozone (O_3), PM_{10}, and carbon monoxide (CO) during the Olympic Games compared with the weeks before and after. However, in a follow-up study of a larger geographic area around Atlanta that examined hospital visits in the years before and after the Olympics, Peel and colleagues did not find any decrease in pediatric emergency room visits (Peel et al. 2010). They did confirm decreased pollutant concentrations during the Games as reported by Friedman and colleagues (2001), but noted similar reductions in O_3 concentrations in several other cities throughout the southeastern United States that were not affected by the traffic intervention. These findings are discussed further in the section "Health Review Committee's Evaluation of the Study."

Temporary controls on traffic were also implemented during the 2002 Asian Games in Busan, Korea. Air pollution levels were measured during the Games and compared with measurements taken in the weeks before and afterward. Lee and colleagues (2007) reported small reductions in air pollutant concentrations during the Asian Games, as well as some beneficial effects on respiratory hospital admissions in children.

Two additional studies evaluated changes in pollutant concentrations resulting from temporary changes in traffic levels: Levy and colleagues (2006) found no evidence for a reduction in the average concentrations of pollutants in a study of temporary road closings aimed at reducing traffic levels around a political event in Boston, Massachusetts. Yuval and colleagues (2008) found that levels of PM, hydrocarbons, and NO_2 dropped considerably in Haifa, Israel, during a military conflict lasting about a month — the conflict disrupted all normal commercial and personal activities.

Factory Closings

Other temporary interventions unrelated to traffic changes have also been studied. A key set of studies evaluated the effects of the temporary closing and reopening of a steel mill that was a major source of emissions in the Utah Valley. The mill closing was associated with decreased morbidity (e.g., fewer hospital admissions for pneumonia and other respiratory diseases) and daily mortality in the area (Pope 1996). In addition, particles collected when the mill was open and when it was closed had different compositions and different biologic effects: specifically, particles collected when the mill was open had higher metal content (particularly, iron, zinc, copper, nickel, and lead) and had greater inflammatory effects both in vivo and in vitro (Ghio 2004).

In addition, Pope and colleagues (2007) examined the effects of an 8-month strike at copper smelters in the southwest United States from 1967 to 1968. They found that over the strike period the concentration of sulfate (SO_4^{2-}) decreased approximately 60% and was accompanied by a decrease in mortality of 2.5%.

In summary, the intervention studies conducted before the current one suggested that Zhang's hypothesis was reasonable — that is, that controls imposed on traffic levels and industrial and commercial activities could be anticipated to affect both air quality and acute or short-term markers of health effects.

TECHNICAL EVALUATION OF THE STUDY

OBJECTIVES AND SPECIFIC AIMS

Zhang and colleagues' study had the following aims:

- **Aim 1:** To estimate exposure of the study subjects to ambient air pollution before, during, and after the

Olympics, and to quantify the change in exposure to each pollutant from before to during the Olympics. Zhang and colleagues also examined whether the post-Olympics pollutant concentrations would return to pre-Olympics levels.

- **Aim 2:** To examine the reversibility of changes in biomarker levels in the study subjects from before to during and from during to after the Olympics. The investigators hypothesized that biomarkers of pulmonary and systemic inflammation, oxidative stress, autonomic dysfunction, and coagulation (including platelet activation) would change significantly during the Olympics air pollution reduction period compared with the pre-Olympics period, and would revert to pre-Olympics levels following relaxation of the air pollution controls after the Olympics.
- **Aim 3:** To examine associations between individual biomarkers and pollutant species across the entire study period and to estimate the unit change in biomarker level per unit change in the concentrations of individual pollutants. The investigators hypothesized that changes in specific pollutants, such as $PM_{2.5}$, gaseous pollutants, and certain PM constituents, would be associated with changes in specific biomarkers.

When the study, referred to as the "Health Effects of an Air Pollution Reduction Trial" (HEART), was under way, Zhang notified HEI that he had also received funding from the National Institute of Environmental Health Sciences (NIEHS). This additional funding — primarily for gene–environment studies that are not part of the current report — allowed Zhang and colleagues to study more subjects and to measure additional endpoints, including markers of platelet activation and urinary 8-OHdG.

STUDY DESIGN

Overview

The study took place from June 2 to October 30, 2008, and was divided into three periods: *pre-Olympics* (June 2–July 20), *during Olympics* (July 21–September 19), and *post-Olympics* (September 20–October 30). The investigators made daily pollution measurements and reported results from 125 healthy, young subjects for the study. Vital signs and a set of biomarkers were measured in each study subject at each of six clinical visits — two within each period.

Air Pollution and Weather Measurements

Zhang and colleagues made measurements of pollutant levels on the roof of a seven-story building located in the center of the Peking University First Hospital campus (see the Commentary Figure). Apart from the measurements of particle number concentration (discussed later in this section), all air samplers and monitors were located at this site. Real-time monitors were operated continuously throughout the entire study and measurement period. The investigators collected samples and measured levels of the following:

- *$PM_{2.5}$ mass concentration:* Measurements were made (in 24-hour samples) both gravimetrically using Teflon filters and in real time using a tapered-element oscillating microbalance (TEOM) monitor. The two methods were highly correlated (see Figure 5 of the Investigators' Report), but as reported in other studies (e.g., Schwab et al. 2006), the TEOM method somewhat underestimated $PM_{2.5}$ mass concentration. Therefore, gravimetrically derived data were the primary source of data for all analyses. On the few dates (n = 6) when gravimetric data were missing, the investigators used an equation (see Figure 5 of the Investigators' Report) to normalize the TEOM data to make them comparable to the gravimetric data.
- *Inorganic ions:* Aqueous extracts of Teflon filters in one of the four-channel $PM_{2.5}$ samplers were analyzed for water-soluble ions F^-, Cl^-, NO_3^-, SO_4^{2-}, NH_4^+, Ca^{2+}, Na^+, Mg^{2+}, and K^+ by ion chromatography.
- *Trace elements:* Extracts of $PM_{2.5}$ prepared from Teflon filters used in another channel of the $PM_{2.5}$ sampler were analyzed by inductively coupled plasma mass spectrometer for the transition metals Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Cd, Th, and U, as well as Na, Mg, Al, P, K, Ca, As, Se, Ba, Tl, and Pb.
- *Elemental carbon (EC) and organic carbon (OC):* $PM_{2.5}$ collected on quartz filters was used to determine OC and EC in an OC/EC analyzer, following the standard protocol of the National Institute for Occupational Safety and Health Reference Method #5040.
- *Particle number:* The investigators made most measurements of particle number concentration at the main campus of Peking University, about 7 km from the hospital where the other pollutant measurements were made. This was done because technical problems with the scanning mobility particle sizer at the Peking University First Hospital site prevented the investigators from measuring particle number concentration there on most of the study days. On the 27 study days on which particle sizers at both sites were functioning, particle number concentration measured at the hospital was about half that measured at the distant

Commentary Figure. Map of Beijing, showing Peking University First Hospital and the 2nd through 6th Ring Roads.

main-campus site. Whether this was due to inherent differences between the two types of instruments or to real differences in pollutant levels at the two sites was not evaluated.

At the Peking University main campus site, the investigators used a twin differential mobility particle sizer to measure particle number concentration at 10-minute intervals (for 24 hours) in 26 size bins with the overall range of 13 to 765 nm. Zhang and colleagues analyzed size-resolved concentration data to determine particle size distributions by period. However, because of time and resource constraints, they analyzed only total particle number (TPN) concentration in relation to biomarkers. Both particle number concentration and TPN provide measures of the number of particles, which are dominated by those in the smallest size fraction of $PM_{2.5}$, namely, ultrafine particles (<100 nm in aerodynamic diameter).

- *Polycyclic aromatic hydrocarbons (PAHs):* Particle-phase PAHs were collected on quartz-fiber filters and extracted into dichloromethane. Zhang's team then measured 15 PAHs by gas chromatography–mass spectroscopy.

- *Gaseous pollutants:* The investigators measured levels of O_3, CO, SO_2, NO, NO_2, and NO_x at the central site. O_3 levels were measured both as 24-hour averages and 1-hour maxima.
- *Meteorologic parameters:* Ambient temperature and relative humidity (RH) were monitored at the central site. The investigators obtained precipitation data from a publicly available Web site that reported rainfall data for Beijing.

Participants

The investigators enrolled 128 healthy subjects — primarily medical residents at Peking University First Hospital — into the study. Eligibility criteria for inclusion in the study included not to have smoked for at least the past year and to be free of any chronic respiratory, cardiovascular, liver, renal, or hematologic diseases, or diabetes mellitus and other systemic diseases. To establish that these criteria were met, each participant completed a medical history before the study started and underwent a physical examination that included routine blood chemistry, spirometry, and electrocardiography (ECG).

Study data are based on 125 subjects (62 women and 63 men, 19–33 years old); 119 completed all 6 clinical visits, and 6 attended 5 visits. Basic demographic information for the study subjects is summarized in Table 1 of the Investigators' Report.

Most of the medical resident participants (105) lived in dormitories, with no cooking facilities, belonging to Peking University Health Sciences Center, located about 5 km from the hospital. The remainder of the participants lived either in dormitories located on the hospital grounds or off campus in nearby areas. All the medical residents studied and worked in the hospital.

Clinical Visits

The study physician supervised the clinical visits, which were conducted at the First Hospital and lasted approximately 1 hour. For each study subject, visits took place on the same day of week as far as was possible and were separated by at least 1 week. Participants were asked to fast on each clinical visit day and not to use aspirin or nonsteroidal anti-inflammatory medications for 2 weeks before testing; these steps were taken to reduce possible effects on platelet activation. The participants were also told not to use anti-inflammatory medications for allergies or other respiratory conditions for 2 weeks before each visit. Visits were rescheduled if the participant had either an active upper-respiratory illness (either infection or allergy) or symptoms of one in the previous 7 days; rescheduling was needed for only 2 subject-visits during the study.

Outcome Measurements

The following measurements were made during each clinical visit:

- ECG (supine position), providing data on heart rate (HR) and heart rate variability (HRV);
- systolic (SBP) and diastolic (DBP) blood pressure;
- biomarkers of systemic inflammation and oxidative stress: white blood cell (WBC) counts and differential cell counts in plasma, as well as levels of fibrinogen, C-reactive protein (CRP), and urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG);
- biomarkers of pulmonary inflammation and oxidative stress: fractional exhaled nitric oxide (FeNO) and exhaled breath condensate (EBC) — specifically, pH (from which they calculated hydrogen ion concentration), nitrate, nitrite, nitrite+nitrate, and 8-isoprostane; and
- biomarkers of plasma clotting pathways: platelet activation (soluble P-selectin [sCD62P] and soluble CD40 ligand [sCD40L]), platelet aggregation, and von Willebrand factor (vWF).

In addition to the above markers of systemic inflammation, peripheral blood was also taken to provide a measure of red blood cell (RBC) count; blood was drawn without using a tourniquet to minimize vascular trauma. Details of the assays for each biomarker are provided in Table 2 of the Investigators' Report and accompanying text.

STATISTICAL ANALYSES

The authors used descriptive statistics, graphs, and box plots of pollutant levels and biomarker data to examine distributions and correlations among the data for the entire study and for each study period. Values below the detectable limit were set to half the detection limit for these calculations.

Zhang and colleagues used time-series regression models to assess the significance of any differences in levels of pollutants between the periods. They included an autoregressive moving-average model structure to control for the non-independence of pollution levels observed on consecutive days and during short time periods and analyzed the data using software that would accommodate the time gaps between periods.

Biomarker data were evaluated as continuous response variables, except for CRP and EBC 8-isoprostane, which were treated as dichotomized variables because several measurements were below the detection limit. Because values for the biomarkers EBC nitrite, EBC nitrate, FeNO, and all HRV measures showed right-skewed distributions,

they were log-transformed before being used in the models. Biomarker values that were greater or less than three standard deviations from the mean were removed as outliers. Correlations between repeat biomarker values in individual subjects were accounted for using a random effect for subject and correlation structures that minimized the Akaike's information criterion. The effect of period was examined by adding indicator variables to models with and without adjustment for temperature and RH (using natural splines of same-day up to 7-day moving averages), sex, and day of the week.

The investigators used these mixed model time analyses to examine associations between daily pollutant and biomarker levels in the more conventional time-series analysis. These models did not include period indicators, but instead incorporated pollution levels on the day of the biomarker measurement and each day up to six days before the biomarker measurement (that is, up to lag day 6).

Pollutant–biomarker analyses with EBC 8-isoprostane were also conducted using hierarchical logistic regression with random effects for subject, controlled for temperature and RH using natural splines, sex, and day of the week. CRP data were not of sufficient quality to merit this additional analytical approach.

Zhang and colleagues also conducted sensitivity analyses for the biomarker differences by period and associations with daily pollution. The analyses compared the results from the models described above to models without adjustments for temperature, RH, and their moving averages; models where observations for days with >1 mm precipitation were removed; and two-pollutant models that used lags with the lowest *P* values (days before biomarker measurement) from the single-pollutant analyses. To adjust for the effect of seasonality across the three periods that was not controlled by the moving averages of temperature and RH (such as the effects of allergens or community infection patterns), the investigators constructed models with indicator variables for period and models excluding the post-Olympics period. For selected biomarkers, the investigators also compared the models with a random effect for subject with exploratory models using a fixed effect.

KEY FINDINGS

Changes in Pollutant Levels

Commentary Table 1 (based on data in Table 4 of the Investigators' Report) shows the percent change in pollutant levels comparing the pre- to during-Olympics periods, and the during- to post-Olympics periods.

Pre- to During-Olympics Comparisons During the Olympics the mean concentrations of all measured pollutants, except O_3, *decreased* compared with the pre-Olympics period concentrations. The decreases in concentration of SO_2, CO, and NO_2 were statistically significant, but the reductions in mean concentrations of $PM_{2.5}$, EC, TPN, SO_4^{2-}, and OC were not. By contrast, O_3 levels (expressed either as a 24-hour average [shown in Commentary Table 1] or 1-hour maximum [see Table 4 of the Investigators' Report]) increased during the Olympics.

During- to Post-Olympics Comparison In the post-Olympics period, the mean concentrations of most pollutants *increased* compared with the during-Olympics period, except for SO_4^{2-} and O_3, both of which decreased. Levels

Commentary Table 1. Mean Pollutant Concentrations and Percent Change in the Three Study Periods[a,b]

Pollutant	Pre- to During-Olympics Mean Concentration (% Change)	During- to Post-Olympics Mean Concentration (% Change)
$PM_{2.5}$ (μg/m^3)	98.9–71.9 (−27.3)	71.9–85.3 (+18.6)
SO_4^{2-} (μg/m^3)	26.5–23.0 (−13.2)	23.0–13.7 (−40.0)
EC (μg/m^3)	2.2–1.4 (−36.4)	**1.4–3.4 (+143)**
OC (μg/m^3)	8.8–6.8 (−22.7)	**6.8–15.0 (+121)**
SO_2 (ppb)	**7.45–2.97 (−60.1)**	**2.97–6.81 (+129)**
CO (ppm)	**1.23–0.64 (−48.0)**	0.64–0.81 (+27.0)
NO_2 (ppb)	**25.60–14.61 (−42.9)**	**14.61–41.39 (+183)**
TPN (/m^3)	16,480–12,853 (−23.7)	**12,853–19,477 (+51.5)**
O_3 (ppb)	31.84–39.60 (+24.4)	**39.60–15.12 (−61.9)**

[a] "+" indicates an increase and "−" a decrease of the percent change in pollutant concentrations between periods.

[b] Boldface indicates statistically significant differences between periods; comparisons of significance were not made for the post- to pre-Olympics period values.

of EC, OC, SO_2, and NO_2 more than doubled. The changes were not statistically significant for $PM_{2.5}$, SO_4^{2-}, or CO (see Figure 6b of the Investigators' Report).

Changes in Particle Composition and Size Distribution An assessment of the relative contributions of $PM_{2.5}$ species as a percentage of total $PM_{2.5}$ mass by period (see Figure 9 of the Investigators' Report) showed that few of the components of PM changed during the Olympics, compared with pre-Olympics contributions. However, there were large changes in PM composition after the Olympics: the proportions of OM, EC, and "other ions" doubled, and transition metals and nitrate increased by about 60% and 40%, respectively, but the proportions of NH_4^+ and PAHs both decreased by about 50%.

Considering all the size ranges of particles measured throughout the entire study, particles with diameters in the range of 108 to 127 nm showed the biggest decrease in concentration in the during-Olympics period.

Changes in Temperature and RH Mean temperature and RH were similar in the pre-Olympics (25.1°C and 66.6% RH) and during-Olympics (27.7°C and 64.8% RH) periods, but both temperature and RH decreased after the Olympics (16.8°C and 48.6% RH). These averages were not unexpected because the pre- and during-Olympics periods fell during the summer (June to September) and the post-Olympics period in the fall (October). However, some during-Olympics days were rainy and had temperatures below the average (T. Wang et al. 2010).

Correlations Among Pollutants Throughout the study period, several pairs of pollutants were moderately to highly correlated (range, r = 0.67–0.92). These included $PM_{2.5}$ mass and its constituents SO_4^{2-}, EC, and OC, but *not* TPN (over the total size range, 13–765 nm diameter). $PM_{2.5}$ mass was similarly highly correlated with SO_2 and CO, but weakly correlated with NO_2 and O_3. O_3 was weakly correlated with SO_2, PM, and SO_4^{2-}, but negatively correlated with NO_2, EC, OC, TPN, and CO. Pairwise correlations among the other gaseous pollutants — SO_2, NO_2, and CO — were weaker ($r < 0.64$).

Changes in Biomarkers Across Periods

Pre- to During-Olympics Comparisons Commentary Table 2 summarizes the statistically significant changes seen in biomarkers by period. During the Olympics, levels of several markers *decreased* compared with pre-Olympics levels (see also Table 6 of the Investigators' Report). The biggest percentage decreases were observed in FeNO

Commentary Table 2. Mean Biomarker Levels and Percent Change Between Study Periods[a]

Biomarker	Pre- to During-Olympics Mean Level (% Change)	During- to Post-Olympics Mean Level (% Change)
FeNO (ppb)[b]	**11.53–4.58 (−60.3)**	**4.58–10.52 (+130)**
8-OHdG (mg/mol creatinine)[b]	**2.16–0.90 (−58.3)**	**0.90–3.74 (+315)**
EBC pH and EBC hydrogen ion concentration[c]	**7.41–7.68 (+3.5, equivalent to −46 hydrogen ion concentration)**	**7.68–7.29 (−4.8, equivalent to +146 hydrogen ion concentration)**
EBC nitrite (μM)[b]	**6.30–4.41 (−30.0)**	**4.41–11.43 (+159)**
EBC nitrate (μM)[b]	**2.84–2.23 (−21.5)**	**2.23–5.82 (+161)**
EBC nitrite+nitrate (μM)[b]	**10.11–8.34 (−17.6)**	**8.34–18.70 (+124)**
SBP (mmHg)	102.5–100.9 (−1.8)	**100.9–110.5 (+10.7)**
HR (bpm)[b]	**66.5–65.4 (−1.7)**	65.4–66.1 (+1.1)
sCD62P (ng/mL)[b]	**6.29–4.16 (−34.0)**	**4.16–5.56 (+33.7)**
sCD40L (ng/mL)[b]	**1.86–1.76 (−5.7)**	1.76–1.92 (+9.1)
vWF (%)	**106.4–92.6 (−13.1)**	92.6–79.5 (−14.2)
Platelet aggregation (%)	**69.29–76.93 (+7.4)**	**76.93–31.65 (−40.8)**
RBC count ($\times 10^{12}$/L)	**4.57–4.61 (+0.9)**	**4.61–4.48 (−2.7)**
WBC count ($\times 10^9$/L)	5.29–5.40 (+0.02)	5.40–5.21 (−0.04)
Lymphocyte count ($\times 10^9$/L)	1.66–1.70 (+0.02)	**1.70–1.59 (−0.07)**

[a] Boldface indicates statistically significant differences between period means.
[b] Geometric means reported because data distributions were skewed.
[c] Calculated by the investigators from the change in pH.

(60.3%), 8-OHdG (58.3%), EBC pH (which increased +3.5%, but corresponding to a decrease in hydrogen ion concentration of 46%), sCD62P (34%), EBC nitrite (30.0%), EBC nitrate (21.5%), EBC nitrite+nitrate (17.6%), and vWF (13.1%). Smaller decreases were also seen in HR (1 bpm, or 1.7%), SBP (1.6 mmHg, or 1.8%), and levels of sCD40L (5.7%). However, some markers — platelet aggregation (+7.4%) and RBC count (+0.9%) — showed unexpected small increases. No significant changes were found in any HRV measurement or in DBP.

During- to Post-Olympics Comparisons Post-Olympics mean concentrations of several markers (that had decreased in the during-Olympics period) *increased* statistically significantly compared with during-Olympics levels: 8-OHdG (315%), FeNO (130%), EBC nitrite (159%), EBC nitrate (161%), EBC nitrite+nitrate (124%), sCD62P (33.7%), EBC pH (−4.8%, but corresponding to an increase in EBC hydrogen ion concentration of 146%). SBP also increased (10.7%). Levels of fibrinogen, sCD40L, and neutrophil count also increased (4.3%, 9.1%, and 4.7%, respectively), but these changes were not statistically significant. Platelet aggregation, which had increased during the Olympics, decreased significantly after the Olympics (41%).

Associations Between Changes in Biomarkers and Individual Pollutants

Single-Pollutant Analyses Changes in individual cardiovascular biomarkers were associated with several pollutants evaluated individually, including $PM_{2.5}$, several $PM_{2.5}$ components, and gaseous pollutants (see Figures 11–30 and Table 8 in the Investigators' Report). Changes generally were observed on multiple lag days after adjusting for meteorology; that is, changes were associated with pollutant levels measured, for example, on the day of a clinical visit (lag 0) or one of several days before the visit (lags 1–6).

The associations were consistent with findings from the period comparisons described above (i.e., changes in most endpoints were *positively* associated with levels of most pollutants except O_3). However, as was seen in the period analyses, unexpected increases in platelet aggregation and RBC counts during the Olympics were *negatively* associated with multiple pollutants, except O_3. Where an association between O_3 and a particular endpoint was observed (e.g., for sCD62LP and 8-OHdG) that association was usually opposite in direction to the association of the same endpoint with the other measured pollutants.

Two-Pollutant Analyses In the two-pollutant analyses, the second pollutant was included in the model at the lag day of its maximum effect on the biomarker (see Appendix C of the Investigators' Report). The investigators indicated that for most of the biomarkers examined, adjusting for a second pollutant resulted in only small changes in individual pollutant–biomarker effect estimates, compared with those changes in the single-pollutant model analysis.

The Committee agreed that this was the case for some biomarkers, for example, EBC nitrite and sCD40L (see Figures C.18 and C.24, respectively, in the Investigators' Report), but there were multiple examples in which the inclusion of a second pollutant substantially attenuated or even changed the direction of the effect of the first; for example, for vWF level, $PM_{2.5}$ reduced the effect of NO_2 to nonsignificance (see Figure C.26), and for sCD62P, CO changed the direction of the effect of EC (see Fig C.23).

Sensitivity Analyses Several different types of sensitivity analyses related to meteorology were conducted. When the investigators deleted nonsignificant temperature and RH results from the models, the overall pollutant–biomarker pattern in the results remained very similar for most of the biomarkers, with a few notable exceptions, such as DBP and SPB (see Appendices J and K in the Investigators' Report). Using the period analysis, the investigators found a change only in the statistical significance but not in the direction for LF (HRV) from the pre- to during-Olympics periods and increases in estimated changes for some HRV indices (HF, LF, SDNN, and total power) from the during- to the post-Olympics period. As they expected, when temperature or RH had a significant effect on a biomarker, the investigators found that the effect estimates from temperature- and RH-adjusted analyses were typically smaller and/or less likely to be statistically significant compared with the effect estimated from nonadjusted analyses. Excluding rainy days from analyses did not change the results substantially, but standard errors of the estimates were generally larger, as expected, due to the reduction in sample size.

Excluding the post-Olympics period (see Appendix M of the Investigators' Report) — because it was essentially a fall rather than summer period and thus likely to represent the largest difference in seasonality — for the selected biomarkers did not change the main findings, except for EBC nitrite; however, this change was still not large enough to alter the overall finding on this biomarker.

By including the "period" indicator as a covariate in the single-pollutant models, the investigators assessed the within-period effects of single pollutants on selected biomarkers (see Appendix N of the Investigators' Report).

Generally, reduced "within-period" effect estimates were found compared with the effect estimates when "period" was not adjusted, except for EBC nitrite+nitrate for which this difference was less notable.

Analysis using subject identification as a fixed rather than a random effect (see Appendix O of the Investigators' Report) showed that the effects of pollutants on biomarkers were attenuated. However, the trend remained largely the same, compared with the effects estimated from the primary models using subject identification as a random effect.

An additional sensitivity analysis including only the 105 subjects who lived in dormitories at Peking University Health Sciences Center (about 5 km away from the hospital) found little effect on the results.

HEALTH REVIEW COMMITTEE'S EVALUATION OF THE STUDY

GENERAL COMMENTS

In its independent review of Zhang and colleagues' study, the HEI Health Review Committee considered it an important contribution to the field of intervention studies; it is one of the first and to date the most comprehensive to evaluate changes in biologic endpoints associated with steps taken to reduce air pollution around the 2008 Beijing Olympics. The study also made important contributions to understanding the effects on cardiovascular endpoints of short-term exposure to air pollutants: In a well-characterized group of healthy medical residents, living close to the hospital in which they worked in Beijing, Zhang and colleagues evaluated the associations of changes in levels of air pollutants with a selection of cardiovascular biomarkers in pathways considered relevant for understanding the pathophysiologic mechanisms of the effects of air pollutants.

The investigators capitalized on the large changes in air pollution levels to conduct an analysis by period (pre-, during- and post-Olympics) to assess whether biomarkers were associated with those changes. A more traditional time-series analysis, focusing on very proximate (within a few days) pollution–biomarker associations, gave a somewhat complementary perspective of the data. Zhang and colleagues conducted appropriate sensitivity analyses to further support their interpretation of the data. The exposure assessment for multiple pollutants was also well designed and well carried out. In the following sections, the Committee discusses and interprets the key findings of the study.

DURING-OLYMPICS CHANGES IN POLLUTANT LEVELS

In reviewing the study, the Committee concluded that Zhang and colleagues conducted a well-designed monitoring scheme that documented changes in levels of many individual air pollutants that were consistent with the effects of a successful intervention. However, the Committee noted that Zhang and colleagues did not set out to identify the extent to which the control measures per se could be considered causal in producing the changes in ambient pollutant levels. For this reason, the changes in the biomarkers could not be directly attributed to the measures taken in the intervention.

Relative to the preceding period, large during-Olympics decreases were found for the pollutant gases SO_2 (60%), CO (48%), and NO_2 (43%) and the particulate pollutants EC and TPN (36% and 24%, respectively). In contrast to the other pollutants, O_3 levels increased 24% during the Olympics. However, the Committee noted that, although mean $PM_{2.5}$ levels dropped by 27% — from 98.9 to 71.9 $\mu g/m^3$ — this change was not statistically significant, and neither were changes in concentrations of SO_4^{2-} and OC. The Committee also noted that both the pre- and during-Olympics concentrations of $PM_{2.5}$ were much higher than concentrations found in most cities outside Asia. Consistent with the lack of significant change in $PM_{2.5}$ concentrations in the current study, compositional analysis indicated few pre- to during-Olympics changes in the proportions of $PM_{2.5}$ constituents.

In general, the changes in pollutant levels recorded by Zhang and colleagues were consistent with changes reported in other studies in Beijing during the Olympics. These studies are summarized in Commentary Table 3 and discussed in more detail in this and subsequent sections.

Zhang and colleagues indicated that the overall pollutant findings were not unexpected, given that they anticipated local motor vehicle emissions would be the dominant pollutant source at the hospital site at which they conducted the monitoring. Thus, their results were consistent with the hypothesis that controls imposed during the Olympics would have a large effect on concentrations of the traffic-associated gaseous pollutants NO_2 and CO, as well as on the particulate components EC and TPN, which can also be considered general markers of traffic-related PM.

The Committee generally agreed with the investigators' interpretation of changes in traffic-related pollutants and noted that the change in $PM_{2.5}$ mass concentrations is consistent with the regional nature of PM concentrations and the fact that PM mass includes both primary PM (which likely decreased during the Olympics) and secondary PM (which, as reported by T. Wang and colleagues [2010], did

Commentary Table 3. Studies of Air Pollution in Beijing During and Surrounding the 2008 Olympics[a]

Study	Measurement Time Period	Monitoring Sites	Key Results
M. Wang et al. (2009)	Pre-control period (starting on July 18) Control period (August 8–23) After the control period ended (September 20 to Oct 6)	4th Ring Road, afternoon	↓ by 40–70% in emissions of CO, NO_x, and SO_2, as well as in benzene, toluene, ethyl benzene, and xylenes from the pre-control period to the Olympic period. ↓ benzene and toluene during the Olympics; smaller ↓ in black carbon and the surface area of ultrafine particles (PM_1). ↑ in all these pollutants in the post-control period.
S. Wang et al. (2010)	Before the Olympic Games (June 2008) During the Olympic Games (from July to August 2008)	Used an emissions inventory model to estimate anthropogenic emissions	↓ 40–60% in the during-Olympics period of daily emissions of SO_2, NO_x, PM_{10}, and volatile organic compounds. Estimated that closing facilities producing construction materials ↓ SO_2 emissions by 85% (48% of total SO_2 emissions), implementing controls on mobile sources ↓ NO_x by 46% and non-methane volatile organic compounds by 57%, and implementing prohibitions on building construction ↓ that sector's PM_{10} emissions by 90% and total PM_{10} by 35%.
T. Wang et al. (2010)	Before the full-scale control (July 11–19) After the full-scale control but before the Olympics (July 20 to August 8) During the Olympics (August 9–24)	Three sites in and outside of Beijing	↓ NO_x and volatile organic compounds during the first two weeks of the control period. ↑ O_3, SO_4^{2-}, and nitrate concentrations increased. ↑ in secondary pollutants after start of full control associated with lack of rainfall and prevalence of southerly winds.
W. Wang et al. (2009)	For on-road measurement, two days during the Traffic Control (TC II) Olympic days (August 12 and 13, 2008) TC-II non-Olympic days (July 25 to August 8 and August 25 to September 20, 2008), TC-II Olympic days (August 9–24, 2008), post-Olympic days with no traffic control (September 20 to October 2, 2008)	Peking University	↓ $PM_{2.5}$ (of 31%) and PM_{10} (35%) in during-Olympics levels; however, some days PM_{10} levels were very high during the control period, but comparatively low on days outside the control period. Using a multivariable linear regression model, estimated that meteorologic factors (including air masses from the south and precipitation), accounted for 40% of total variation in PM_{10} concentrations, whereas source control measures accounted for only 16%.
Lin et al. (2011)	June 11–22, 2007; September 10–20, 2007; December 10–21, 2007; June 16–27, 2008; September 1–12, 2008	Peking University campus	↓ black carbon (by 64%) and $PM_{2.5}$ (70%) during the traffic intervention (July 20 to September 17). ↓ exhaled NO (by 27%) in Beijing school children.
Wang and Xie (2009)	"Traffic Demand Management" days, starting July 20, 2008, as compared with pre-"Traffic Demand Management" days	12 streets that formed urban street canyons on different sides of Beijing's 2nd, 3rd, and 4th Ring Roads during the time period in which vehicle operation on odd or even days was enforced	↓ traffic flow (32.3%); using a model to predict pollutant concentrations on those streets during "Traffic Demand Management" days, ↓ in concentrations of PM_{10} (28%), CO (19%), and NO_2 (12%), while ↑ in concentrations of O_3 (25%).
X. Wang et al. (2009)	TC-II non-Olympic days (July 25 to August 8 and August 25 to September 20, 2008), TC-II Olympic days (August 9–24, 2008), post-Olympic days with no traffic control (September 20 to October 2, 2008) On-road measurements performed on two days during TC-II Olympic days (August 12 and 13, 2008)	Peking University Health Science Center campus, 6 m and 20 m above ground. On-road site: 4th Ring Road and Badaling Expressway in North Beijing	↓ by 74% in median ambient black carbon concentration on traffic control days during Olympics compared with non-traffic-control days in 2008. ↓ emission factors for black carbon, CO, and ultrafine particles for many types of vehicles in 2008 compared with 2007.

[a] ↑ and ↓ signify increase and decrease in concentration, respectively.

not change much and might even have increased during the Olympics).

However, the Committee noted that a crucial difference between the Beijing Olympics restrictions and prior traffic intervention studies (e.g., Friedman et al. 2001; Hugosson et al. 2006; Peel et al. 2010; Kelly et al. 2011) is that the restrictions imposed in Beijing were much more intensively aimed at reducing traffic, while at the same time including restrictions on a wider range of pollutant sources than just traffic. Severe restrictions were imposed on operations within and around the city at power plants and construction sites, which were both expected to generate substantial pollutant emissions. Thus, larger changes in $PM_{2.5}$ and other pollutants might have been expected. The additional restrictions, rather than the controls on traffic, were also likely to have been responsible for the large decrease in SO_2 concentrations found in the current study.

As in other studies conducted in Beijing in this period (Wang and Xie 2009; T. Wang et al. 2010), Zhang and colleagues' study reported an increase in O_3 concentrations during the Olympics. Zhang and colleagues attributed this increase to the decrease in concentrations of NO_2 and, more specifically, NO, which are known to titrate O_3 and thus reduce monitored concentrations of O_3 when they are present.

Comparison of Pollutant Findings in the Current Study with Other Beijing Olympics Studies

Overall, the magnitude of the changes in pollutant levels associated with the during-Olympics period described by Zhang and colleagues is consistent with results of other studies (see Commentary Table 3) conducted in and around Beijing at the same period (M. Wang et al. 2009; W. Wang et al. 2009; X. Wang et al. 2009; S. Wang et al. 2010; T. Wang et al. 2010; Lin et al. 2011.) In summary, these studies found during-Olympics decreases in many primary pollutants, especially those associated with traffic, but found increases in O_3 concentrations as well as some measures of secondary pollutants. Note that Commentary Figure 1 shows the location of the Peking University Hospital (the central monitoring site) and Beijing's 2nd to 6th Ring Roads — the major traffic routes around the city where changes in traffic-derived air pollution were likely to be most evident.

The reduction in primary, traffic-related PM reported in the current study is supported by X. Wang and colleagues (2009). With monitors at Peking University Health Science Center and at an on-road site on the 4th Ring Road, this study also found a major reduction (74%) in concentrations of black carbon — an indicator of traffic-related air pollution — during the period the traffic intervention was in place compared with when it was not. Similarly, using data from a monitoring site on Peking University campus (a few blocks north of the 4th Ring Road), Lin and colleagues (2011) reported major reductions in black carbon and $PM_{2.5}$ (64% and 70%, respectively) during the traffic intervention (20 July–17 September). Wang and Xie (2009) found a 32% reduction in traffic flow on 12 streets that formed urban street canyons on different sides of Beijing's 2nd, 3rd, and 4th Ring Roads during the time period in which vehicles were restricted by date according to their odd or even license plate numbers (which Wang and Xie labeled "Traffic Demand Management" days [starting July 20, 2008] as compared with pre-Traffic Demand Management days). Using a model to predict pollutant concentrations on those streets during Traffic Demand Management days, they reported concomitant reductions in concentrations of PM_{10}, CO, and NO_2 of 28%, 19%, and 12%, respectively, while O_3 concentrations increased by 25%.

W. Wang and colleagues (2009) found decreases of 31% and 35% in during-Olympics concentrations of $PM_{2.5}$ and PM_{10}, respectively, measured at Peking University, which is located in northwestern Beijing in a primarily residential and commercial area, without major industrial sources. However, they also noted that PM_{10} concentrations on some days during the control period were very high, but then were comparatively low on days outside the control period, suggesting that factors other than the imposed controls were at work.

T. Wang and colleagues (2010) measured pollutant levels at three sites in and outside Beijing and reported that air quality improved significantly during the Olympics, specifically, decreases in concentrations of NO_x and volatile organic compounds (which they associated with a drop in vehicular traffic) during the first two weeks of the control period. However, O_3, SO_4^{2-}, and nitrate concentrations increased.

Using a mobile laboratory to make on-road measurements along the 4th Ring Road, M. Wang and colleagues (2009) found major reductions (by 40–70%) in emissions of CO, NO_x, and SO_2, as well as of benzene, toluene, ethyl benzene, and xylenes (mostly, but not exclusively, derived from traffic) from the pre-control period to the Olympics period; smaller decreases were also found in concentrations of black carbon and the surface area of ultrafine particles ($PM \leq 1$ µm in aerodynamic diameter, or PM_1). Concentrations of all these pollutants increased in the post-control period. The investigators concluded that the changes in pollutant concentrations were most strongly influenced by traffic density during the different periods. A decrease in the benzene-to-toluene ratio during the

Olympics suggested that pre-Olympics painting projects (a major source of toluene) had stopped. M. Wang and colleagues' findings also suggested that SO_2 was derived from both local emissions and regional transport.

These measured changes in ambient pollution levels are supported by the study of S. Wang and colleagues (2010) who reported 40% to 60% reductions in daily emissions of SO_2, NO_x, PM_{10}, and volatile organic compounds during the Olympics compared with June 2008. They estimated that closing facilities that produce construction materials reduced SO_2 emissions by 85% (48% of total SO_2 emissions), implementing controls on mobile sources reduced NO_x and volatile organic compounds by 46% and 57%, respectively, and mandating prohibitions on building construction reduced that sector's PM_{10} emissions by 90% and total PM_{10} by 35%.

CHANGES IN BIOMARKER LEVELS

The Committee agreed with the investigators' conclusions that the analyses by period found multiple changes in levels of cardiovascular biomarkers and that the changes were generally consistent with the investigators' hypotheses — specifically, that when biomarker levels before and during the Olympics were compared, most changes during the Olympics were in a direction reflecting a decrease in adverse effects; in addition, post-Olympics mean concentrations of several biomarkers (which had decreased in the during-Olympics period) were also increased compared with during-Olympics levels.

Large changes were found in some biomarkers representative of different physiologic pathways — coagulation (sCD62P and vWF), airway inflammation (FeNO, EBC hydrogen ion, nitrite, and nitrate), and oxidative stress (urinary 8-OHdG). These changes in biomarkers support the view that exposure to air pollution affects several non-mutually exclusive pathophysiologic pathways: coagulation in the circulation, inflammation in the airways, and the activation of oxidative stress. No associations were found with CRP — a measure of general systemic inflammation — but this may have been due to the poor quality of the CRP data. Zhang and colleagues' findings of during-Olympics decreases in biomarkers of airway inflammation confirm and expand the findings of Lin et al. (2011), who reported during-Olympics decreases in black carbon and $PM_{2.5}$ that were associated with a reduction in eNO in asthmatic and nonasthmatic school children in Beijing (see Commentary Table 3).

The Committee also agreed with Zhang and colleagues that not all the pre- to during-Olympics changes in biomarkers were consistent with a decrease in adverse effects. In particular, levels of platelet aggregation — and, to a lesser extent, WBC and RBC counts — changed in a direction opposite to that expected for a decrease in adverse effects. Even more surprisingly, the direction of change in platelet aggregation was inconsistent with the direction of change in other biomarkers of platelet activation and coagulation — sCD62P and vWF. Interestingly, this difference in direction of change between platelet aggregation and sCD62P was maintained in the post-Olympics period: the direction of the during-Olympics change was reversed for both biomarkers. The Committee agreed with the investigators that there was no obvious way to reconcile these discrepant findings.

Zhang and colleagues' analysis by period did not find effects on markers of change in the autonomic nervous system (HR and indices of HRV), while their time-series analyses (discussed later in the section "Time-Series Analysis: Associations Between Specific Pollutants and Individual Biomarkers") did find some examples of associations between acute changes in HRV and individual pollutants. Thus, these results do not add clarity regarding the role of the autonomic nervous system as a pathway for adverse cardiovascular effects of air pollution.

The Committee noted that these observations were made in young healthy subjects and so may not reflect changes that may be seen in susceptible populations, such as those with pre-existing cardiorespiratory conditions (e.g., asthma or cardiovascular disease) or those with polymorphisms in genes whose protein products are involved in physiologic defenses (e.g., variations in expression of the detoxifying enzyme family of glutathione *S*-transferases, as reported in Gilliland et al. [2004]). The Committee recognized that Zhang and colleagues were funded by NIEHS to evaluate responses in the study subjects who differed in expression of different glutathione *S*-transferases, and that these results would not be part of the current report. In addition, given the study's focus on acute reversible changes in biomarkers of coagulation, inflammation, and oxidative stress after exposure to air pollution, it was not clear whether the changes reported would have any impact, positively or negatively, on disease or adverse outcomes.

The Committee noted that Zhang and colleagues collected information on a relevant suite of cardiovascular biomarkers, but did not take advantage of the multiple clinical visits to measure respiratory function (e.g., through the use of spirometry) or record any clinical symptoms (such as cough, wheeze, etc.) in the study subjects. Although not part of the study design, the Committee thought including these endpoints would have added another set of potentially helpful health data to the study.

Sensitivity Analyses and Possible Residual Biases

The Committee agreed with Zhang and colleagues that the robustness of the results of their sensitivity analyses, which took into consideration several different models for meteorologic effects, reinforced the interpretation that changes in biomarkers were due to the changes in pollution. The fact that the pre- and during-Olympics periods occurred essentially during the same season made the comparison between them particularly strong. However, relying entirely on meteorology to control for seasonal patterns cannot exclude the possibility of confounding by other seasonal factors, for example, community viral infections or aeroallergens. The Committee thought that it was also plausible that other causes or factors during the intervention — such as changes in ambient noise levels or subtle alterations in the lifestyle patterns and stress levels of the subjects in response to the atmosphere surrounding the Olympics — might have contributed to the observed changes in biomarkers.

Time-Series Analyses: Associations Between Specific Pollutants and Individual Biomarkers

Zhang and colleagues had initially hypothesized that specific pollutants would be associated with changes in individual biomarkers. This was not the case, however, as the time-series analyses found that multiple pollutants — $PM_{2.5}$ and its components, as well as the gaseous pollutants — were associated with each biomarker. Generally, but not always, these associations held at multiple lag days (that is, on a specific day [0–6] before the clinical visit).

The Committee considered that the investigators' methods for time-series analysis of pollutant–biomarker associations were generally state of the art, but noted one important exception: there was no direct control for long-term time trends (slow changes over time), which is standard in time-series epidemiology (known as the "time smooth"). The Committee considered that this omission was insufficiently acknowledged by the investigators and complicated the interpretation of the results of this analysis; that is, without the control of slow changes in time, the results from the daily time-series analyses of pollutant–biomarker associations are not independent of the between-period comparisons discussed above. For example, given the clear reductions in many markers of pulmonary oxidative stress from the pre- to the during-Olympics periods and the subsequent increase after the Olympics (after adjusting for covariates), which was mirrored in the pattern for the pollutant concentrations, it would be expected that there would be a clear association between the two. It is thus not surprising that these associations are found at nearly all the lags considered, since all lags will reflect the between-period differences in concentrations.

There are several implications of this. First, the results from the time-series analyses cannot be considered as independent confirmation of those from the between-period analyses. Second, the lag between exposure and change in individual biomarkers cannot be reliably inferred from these analyses, because an acute (lag 0) or delayed (lag 6) effect would tend to show up (whatever the true lag was) when exposure had been stable for a month as effects at all lags 0 to 6. Third, given that many pollutants changed similarly across periods, the ability to discriminate associations of daily levels of each pollutant with biomarkers will be limited. This is confirmed by the high correlations between many specific pollutant concentrations (see Table 5 in the Investigators' Report).

However, the time-series results also draw on between-day within-period associations; the investigators present this component separately (with period effects adjusted out) as sensitivity analyses in Appendix N of the Investigators' Report. These time-series results do give genuinely independent evidence; they also avoid the second problem noted above (the confounding of one lag by others) and, to a somewhat lesser extent, the third problem (correlation between pollutants). On the other hand, they do not exploit the particular feature of this study — the large changes due to the intervention. The period-adjusted pollutant–biomarker associations (shown in Appendix N) were very different from the overall associations, and in many cases, the two different analyses are inconsistent (with non-overlapping confidence intervals), suggesting rather different signals from the within-period and between-period associations. Between-period associations of pollutants with outcomes (e.g., using period mean concentrations as explanatory variables in regressions) are not shown in this report.

These observations suggested to the Committee that caution be exercised with regard to the investigators' conclusion that effects of PM and components at shorter lags were associated with markers of pulmonary changes, whereas effects at longer lags were associated with markers of systemic effect (thus, lending support to the idea that PM effects were observed first in the airways and from there spread through the circulation). Further, the patterns of association between pollutants at different lags and individual biomarkers in the current study, in any case, seemed to show an insufficiently clear pattern from which to draw this conclusion. For example, the positive or negative associations reported between individual pollutants (such as $PM_{2.5}$ and SO_4^{2-}) and multiple systemic biomarkers (particularly, sCD62P, RBC, and 8-OHdG) were all significant on lag day 0.

Associations between O_3 and some biomarkers, particularly sCD62P and vWF, were negative, but it is highly

unlikely that this reflects a protective effect of O_3 because it is known to have multiple acute effects on respiratory and inflammatory endpoints (reviewed in Kim et al. 2011). Plausible explanations of Zhang and colleagues' finding of negative associations between O_3 and cardiovascular biomarkers in the current study are still needed. Given the negative correlation between O_3 and many other pollutants, the odd, negative correlation for O_3 compared with positive associations for many other pollutants may be an illustration of the difficulties in trying to distinguish the effects of one pollutant from another. This issue is also likely to be relevant for other pollutant–biomarker associations.

The Committee commends the investigators for conducting numerous sensitivity analyses. Apart from the period-adjusted analyses (discussed above), they were broadly reassuring that results were not dependent on the specifics of the model chosen.

Lack of Geographic Comparison

The Committee recognized that conducting geographic (or historical) comparisons was not part of the present study and beyond its scope in both scientific and budgetary terms. However, they noted that inclusion of a nearby control area — as similar in characteristics to the area of the intervention as possible, in which exposure was measured and a similar group of participants was followed with the same instruments over the same time period — would have enhanced the ability of the investigators to attribute the changes in pollutant levels and biomarkers to the intervention. Inclusion of such a control would have greatly reduced the limitations of the interpretation of the results that the Committee noted in the current study.

The importance of considering a wider geographic area in interpreting pollution changes in intervention studies was also referred to earlier in this Commentary in the discussion concerning the differences in O_3 findings between the studies by Friedman and colleagues (2001) and Peel and colleagues (2010) from the Atlanta Olympics. These studies illustrate the crucial importance of using relevant comparison areas, evaluating appropriate time windows surrounding interventions, and properly adjusting for seasonal and other trends that may influence the results (see recommendations from the HEI Accountability Workshop addressing these concerns [Health Effects Institute 2010]).

Accuracy of Assessment of Participants' Exposure

The Committee agreed with Zhang and colleagues that the pollutant measurements made at the outdoor Peking University Hospital site were unlikely to accurately reflect the actual level of exposure of the study participants. One reason is that most of them (105 of 125) lived 5 km away from the monitoring site and thus spent several hours of the day at that distance from the monitor. In addition, the participants spent most of their time indoors — Table D.2 of Appendix D of the Investigators' Report indicates over 23 hours per day — and worked in air-conditioned facilities. To what extent this introduced error in the exposure assessment is unknown. However, there was no likely systematic pattern to this type of error, and so this possible error was considered unlikely to have an impact on the effect estimates reported in the current study.

Because the participants spent so much time indoors, they were also likely to be exposed to sources of indoor pollution, as well as pollutants that infiltrated from the outside. Alert to the possibility of potential confounding by indoor sources, the investigators tried to minimize exposure to major indoor sources — cooking and heating — in the study design by selecting study participants who lived in places in which they could not or did not cook (Appendix D of the Investigators' Report indicates that time spent cooking was very low). Nonetheless, the Committee considered it likely that some sources of exposure to pollution — indoor, as well as during the commute to work — had not been captured in the study.

However, for this study, an accurate estimation of absolute (personal) exposures is not essential. What is important is that the changes in these study subjects' exposures over time (between periods and between days) are adequately characterized. Several studies conducted since the 1990s (e.g., Brunekreef et al. 2005) have shown that, especially for fine PM, the temporal correlation between central ambient monitoring and personal exposure is usually considered quite acceptable for meaningful epidemiology.

SUMMARY AND CONCLUSIONS

In its independent review, the HEI Health Review Committee considered the study an important contribution to the literature regarding short-term interventions and their impacts on acute health responses — it is one of the first, and to date the most comprehensive study, to evaluate changes in biologic endpoints associated with the control measures taken to reduce air pollution associated with specific, short term events. The investigators capitalized on the large changes in air pollutants to conduct an analysis by period (pre-, during-, and post-Olympics) to assess whether biomarkers were associated with those changes. A more traditional time-series analysis, focusing on very proximate (within a few days) pollution–biomarker associations

gave a somewhat complementary perspective of the data. The exposure assessment for multiple pollutants was also relatively comprehensive. Apart from measurements of particle number, the measurements of all pollutants were made at a single central Beijing site, close to where the participants lived and worked. Furthermore, in this well-characterized group of healthy subjects, Zhang and colleagues evaluated a representative group of pulmonary, systemic, and urinary biomarkers in pathways considered relevant for understanding the pathophysiologic mechanisms of the effects of air pollutants. Zhang and colleagues also conducted appropriate sensitivity analyses — including adjusting for meteorology and (in the time-series analyses) for an effect of period independent of pollution — to provide further support for their interpretations. Thus, the study represents one of the most comprehensive to date to evaluate the effects of exposure to air pollution on a myriad of potential short-term cardiovascular biomarkers.

Reviewing Zhang and colleagues' comparisons of pollutant concentrations before and during the Olympics, the Committee agreed that the investigators found many during-Olympics changes in pollutant levels and that these were consistent with the effects of a successful intervention. However, the Committee noted that the investigators had not designed the study to identify the extent to which the control measures per se could be considered causal in producing the changes in ambient pollutant levels. Therefore, the changes in the biomarkers they had measured, which were consistent with the measured improvements of air quality, may not be directly attributed to the interventions.

The large during-Olympics decreases (40–60%) found for the pollutant gases SO_2, CO, and NO_2, and for particulate pollutants (EC and TPN, with smaller changes in $PM_{2.5}$, SO_4^{2-}, and OC concentrations) were generally consistent with other studies of pollutant concentrations in Beijing conducted in the time period around the Olympics. The major decrease in during-Olympics concentrations of SO_2 was most likely due to the restrictions imposed on construction and power plant activities in and around Beijing. Decreases in NO_2, CO, EC, and TPN (the latter a marker of ultrafine particles, which dominate this measure) were most likely attributable to restrictions on traffic. After the controls were relaxed in the post-Olympics period, concentrations of most pollutants (except O_3) increased, and changes were seen in PM components that were generally consistent with increases in traffic and other emissions sources.

Changes in the levels of several cardiovascular biomarkers from the pre- to during-Olympics periods were observed, and the Committee agreed with Zhang and colleagues that these changes were generally consistent with the investigators' hypothesis that changes would be in a direction reflecting a decrease in adverse effects. Using both by-period and time-series analyses, the investigators found large changes in some biomarkers in several pathophysiologic pathways through which PM may exert its effects — coagulation in the circulation (sCD62P and vWF), inflammation in the airways (FeNO, EBC hydrogen ion, nitrite, and nitrate), and the activation of oxidative stress (urinary 8-OHdG). However, unexpectedly and without obvious explanation, levels of some biomarkers — in particular platelet aggregation in the coagulation pathway — changed in the opposite direction from other biomarkers in the same pathway. Given the number of observations made in the study, a few might have increased by chance.

The finding that the post-Olympics mean levels of several biomarkers increased compared with during-Olympics levels was largely consistent with the investigators' hypotheses that changes in air pollutant levels in the different periods would be reflected in changes in biomarker levels and that the pre- to during- and during- to post-Olympics changes would be inversely related. It is noteworthy that these observations were made in young healthy subjects and so may not reflect changes that might be seen in susceptible populations, such as those with pre-existing cardiorespiratory conditions (e.g., asthma or cardiovascular disease) or those with variations in genes whose protein products are involved in physiologic defenses. In addition, given the study's focus on acute reversible changes after exposure to air pollution, this study does not shed light on whether these changes would have any impact, positive or negative, on disease or adverse outcomes.

Although the investigators had hypothesized that individual pollutants would be associated with changes in individual biomarkers, multiple pollutants were associated with every biomarker. In hindsight, attributing changes to specific pollutants was likely to be challenging, given that the intervention was multifaceted and affected multiple sources and pollutants.

The multiple sensitivity analyses Zhang's team conducted bolstered the interpretation that the changes in biomarkers were related to changes in levels of air pollution. However, the Committee thought that some caution should be retained in attributing the between-period biomarker differences to pollution, given the possibility that other unmeasured risk factors — such as changes in virus infections, ambient noise levels, or subtle alterations in lifestyle patterns and stress levels in the participants in response to the atmosphere surrounding the Olympics — might have contributed to the differences observed.

Because of the large influence of between-period contrasts in the time-series analyses of the association of specific pollutants with biomarkers, similar caution is needed for these results. The time-series analyses were generally consistent with the between-period analyses, but could not discriminate among the several pollutants, which changed in concert. Exposure misclassification may also have been an issue — that is, pollutant measurements made at the outdoor site may not have accurately reflected the actual level of exposure of the study participants. However, the Committee considered that it was unlikely there was a systematic pattern to this type of error, and so this possible error was considered unlikely to have an impact on the effect estimates reported in the current study.

The Committee suggested that future studies to evaluate the effects of an intervention in a city should include a nearby control area — as similar in characteristics to the area of the intervention as possible — in which exposure would be measured and a similar group of participants would be followed with the same instruments over the same time period. Although the cost of the study would increase substantially, the Committee thought inclusion of such a control would enhance the investigators' ability to attribute the changes in pollutant levels and biomarkers to the intervention and greatly reduce the limitations of the interpretation of the results as noted above. Overall, and despite the challenges in conducting such a study, this study carried out by Zhang and colleagues provides important supporting evidence that air quality improvements such as those found during the Beijing Olympics can improve health biomarkers, with the potential for beneficial health effects in the affected population.

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