Inflammatory and Oxidative Stress Responses of Healthy Young Adults to Changes in Air Quality during the Beijing Olympics

Wei Huang[1]*, Guangfa Wang[2]*, Shou-En Lu[3,4], Howard Kipen[3,5], Yuedan Wang[6], Min Hu[1], Weiwei Lin[1], David Rich[7], Pamela Ohman-Strickland[3,4], Scott R. Diehl[8], Ping Zhu[9], Jian Tong[10], Jicheng Gong[11], Tong Zhu[1], and Junfeng Zhang[10,11]

[1]College of Environmental Sciences and Engineering and Centre for Environment and Health, Peking University, Beijing, China; [2]Department of Pulmonary Medicine and [9]Department of Hematology, Peking University First Hospital, Beijing, China; [3]Department of Environmental and Occupational Medicine and [5]Environmental and Occupational Health Sciences Institute, Robert Wood Johnson Medical School, [4]Department of Biostatistics and [10]Department of Environmental Health, School of Public Health, and [8]Center for Pharmacogenomics and Complex Disease, School of Dentistry, University of Medicine and Dentistry of New Jersey, Piscataway, New Jersey; [6]Department of Immunology, Peking University Health Sciences Center, Beijing, China; [7]Department of Community and Preventive Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York; and [11]Department of Preventive Medicine, University of Southern California, Keck School of Medicine, Los Angeles, California

Rationale: **Unprecedented pollution control actions during the Beijing Olympics provided a quasi-experimental opportunity to examine biologic responses to drastic changes in air pollution levels.**

Objectives: **To determine whether changes in levels of biomarkers reflecting pulmonary inflammation and pulmonary and systemic oxidative stress were associated with changes in air pollution levels in healthy young adults.**

Methods: **We measured fractional exhaled nitric oxide, a number of exhaled breath condensate markers (H^+, nitrite, nitrate, and 8-isoprostane), and urinary 8-hydroxy-2-deoxyguanosine in 125 participants twice in each of the pre- (high pollution), during- (low pollution), and post-Olympic (high pollution) periods. We measured concentrations of air pollutants near where the participants lived and worked. We used mixed-effects models to estimate changes in biomarker levels across the three periods and to examine whether changes in biomarker levels were associated with changes in pollutant concentrations, adjusting for meteorologic parameters.**

Measurements and Main Results: **From the pre- to the during-Olympic period, we observed significant and often large decreases (ranging from −4.5% to −72.5%) in levels of all the biomarkers. From the during-Olympic to the post-Olympic period, we observed significant and larger increases (48–360%) in levels of these same biomarkers. Moreover, increased pollutant concentrations were consistently associated with statistically significant increases in biomarker levels.**

Conclusions: **These findings support the important role of oxidative stress and that of pulmonary inflammation in mediating air pollution health effects. The findings demonstrate the utility of novel and noninvasive biomarkers in the general population consisting largely of healthy individuals.**

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Increased air pollution concentrations have been associated with increased mortality and morbidity. The overlapping mechanisms underpinning pulmonary inflammation and respiratory and systemic oxidative stress in relation to air pollution exposure remain unclear.

What This Study Adds to the Field

We observed significant improvements in biomarkers of pulmonary inflammation and biomarkers of respiratory and systemic oxidative stress during the Beijing Olympic air pollution control period in young healthy subjects. The findings from this real-world study provide quasi-experimental, mechanistic data to support that air pollution adversely affects young and healthy individuals through acute changes in pulmonary inflammation, airway, and systemic oxidative damage. This study also demonstrates the utility of novel and noninvasive biomarkers of pathophysiologic pathways using human breath specimen.

Keywords: air pollution; inflammation; oxidative stress; respiratory health; the Beijing Olympics

Increased air pollution concentrations have previously been associated with increased cardiorespiratory mortality and morbidity (1–5). However, observational and experimental studies in humans or animals have generated limited and somewhat inconsistent data supporting several postulated pathophysiologic pathways (6–10). One of these is the hypothesis that inhaled pollutants can react rapidly with extracelluar macromolecules or cell constituents in the airway epithelium to generate reactive oxygen or nitrogen species (e.g., free radicals and peroxides), inducing local and systemic oxidative or nitrosative stress and subsequent inflammation (11).

Pulmonary inflammation and oxidative stress responses to air pollution have been examined in human studies using several noninvasive biomarkers in exhaled breath and exhaled breath condensate (EBC) (7, 12–18). Increased air pollution levels have been associated with increased levels of fractional exhaled nitric oxide (F_{ENO}), reflecting pulmonary inflammation, in children and the elderly (12–15, 19–22). Traffic pollution exposure has been associated with increased airway acidity (lowered EBC pH) in

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* These authors made equal contributions and serve as co–first authors.

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Correspondence and requests for reprints should be addressed to Junfeng (Jim) Zhang, Ph.D., 2001 North Soto Street, Room 205Q, Los Angeles, CA 90089. E-mail: junfengz@usc.edu

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persons with asthma (16), reflecting inhibition of local epithelial proton pumps during airway inflammation (23). Furthermore, several novel EBC biomarkers reflecting oxidative and nitrosative stress in the respiratory tract (e.g., EBC nitrite, nitrate and 8-isoprostane) have been associated with air pollution exposure (17, 24–26). Recent studies also suggest that an increased whole-body burden of oxidative stress may induce or exacerbate pulmonary or systemic inflammation. These studies often use urinary 8-hydroxy-2-deoxyguanosine (8-OHdG) as a biomarker of systemic oxidative stress (27, 28). However, the overlapping mechanisms underpinning pulmonary inflammation, local (respiratory tract) and systemic (whole-body) oxidative stress, in relation to air pollution exposure, remain unclear. Major obstacles include the lack of "controlled" contrasting exposure conditions and the lack of noninvasive pathway-specific biomarkers that are sensitive to day-to-day changes in air pollution levels, as done in typical panel studies (9, 14, 16).

Unprecedented pollution control actions during the 2008 Beijing Olympics resulted in substantial temporary air quality improvements, providing a quasi-experimental opportunity to assess whether the drastic change in air quality would lead to changes in pathway-specific biomarkers, thereby elucidating mechanisms underlying air pollution health effects. Taking advantage of this opportunity, we have designed a study to examine several prominently hypothesized mechanisms of cardiorespiratory health effects from air pollution, including pulmonary inflammation, systemic inflammation, thrombosis, oxidative stress, and antonomic dysfunction (29). In the present paper, we focus on three interrelated pathways including pulmonary inflammation, pulmonary oxidative and nitrosative stress, and systemic oxidative stress. We measured a suite of noninvasive biomarkers, some of which are novel (e.g., EBC markers), reflecting these pathways in a panel of healthy adults. We hypothesized that each of these biomarkers would improve (reduced level of inflammation and oxidative stress) from the pre-Olympic period (without pollution controls) to the during-Olympic period (with pollution controls), followed by worsening from the during-Olympic period to the post-Olympic period (without pollution controls). We further hypothesized that changes in biomarker levels would be associated with changes in concentrations of pollutants. The results on air pollution associated with systemic inflammation and thrombosis, along with heart rate and blood pressure, have been reported elsewhere (29).

METHODS

Study Design and Participants

This panel study, nested within a quasi-experimental design with "high-low-high" pollution levels, was designed according to the timelines of the Olympic pollution control measures (30). The whole study period included three subperiods: (*1*) the pre-Olympic period (June 2–July 20); (*2*) the during-Olympic period (July 21–September 17) when industrial and commercial combustion facility operation and vehicle use were strictly controlled; and (*3*) the post-Olympic period (September 30–October 30) when the pollution control measures were relaxed (Figure 1). Participants were repeatedly measured for biomarkers twice, at least 1 week apart, within each subperiod.

Participants were a homogenous group of nonsmoking medical residents at Peking University First Hospital located in central Beijing, with regimented work and living lifestyles (29). Enrollment required each participant to complete a medical history, physical examination, routine blood chemistries, spirometry, and electrocardiogram to rule out any medical conditions that would preclude participation. Through on-site advertisements and with informed consent, we screened 137 persons, among which 128 met the inclusion criteria (never smoked and had no chronic diseases) and agreed to participate. Three participants withdrew from the study after taking part in the first one or two clinical visits, and thus were excluded from data analyses. Among the remaining 125 participants, 119 completed all six visits and six participants only missed one visit. These participants (62 female and 63 male, from 19–33 yr old) and their 744 person-visits were used in statistical analyses.

The study was approved by the joint Ethics Committee of Peking University Health Sciences Center and Peking University First Hospital, and the University of Medicine and Dentistry of New Jersey Institutional Review Board.

Clinical Visits and Biomarker Measurements

Participants could not have an active upper respiratory illness (either infection or allergy) and could not use antiinflammatory medication for allergies or other respiratory conditions for at least a week before a clinic visit. Each experimental session was about 60 minutes for each participant, starting with electrocardiogram monitoring in the supine

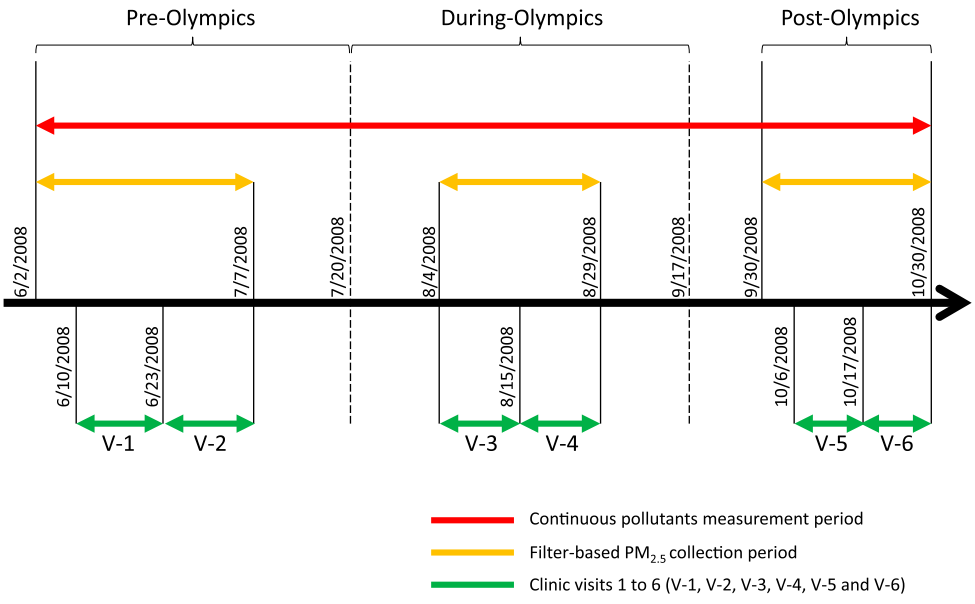


Figure 1. Operational definition of the pre-, during-, and post-Olympic periods in relation to the start and end dates for air pollutant measurements and clinical visits within each period. 0 = 0–23 hours before clinic visit; 1 = 24–47 hours before clinic visit; 2 = 48–71 hours before clinic visit; 3 = 72–95 hours before clinic visit; 4 = 96–119 hours before clinic visit; 5 = 120–143 hours before clinic visit; 6 = 144–167 hours before clinic visit.

position to measure heart rate and heart rate variability. Then we measured blood pressure and the following protocols without a particular order. Fasting blood samples were drawn to measure biomarkers of systemic inflammation and thrombosis, as described previously (29). F_{ENO} and EBC samples were collected to measure for markers of pulmonary inflammation and oxidative stress. A spot urine sample was collected during each visit at a convenient time.

We measured F_{ENO} using an offline sampling method, following the recommendations of the American Thoracic Society/European Respiratory Society (31), which has been described previously (21). All measurements were made with participants being seated, after 3–5 minutes of rest. Participants inhaled from functional residual capacity through a mouthpiece with a NO-scrubber attached, thereby inhaling NO-free air, followed by a controlled expiration through the mouthpiece. A resistive pressure of 13 cm H_2O 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 bronchial epithelial cells into a NO-impermeable aluminum foil bag. The exhaled breath samples were analyzed for F_{ENO} using a $NO/NO_2/NO_x$ chemiluminescence analyzer (Model 42i; ThermoScientific, Rockford, IL) within 3 hours of collection. The analyzer had a detection limit of 0.40 ppb and an accuracy of $\pm$ 0.40 ppb. The analyzer was calibrated daily with five concentrations of NO (ranging from 0–80 ppb).

We collected EBC samples using a Jaeger EcoScreen collector (Erich Jaeger, Hoechberg, Germany). The machine was switched on at least 30 minutes before collection to allow the cooling cuff to 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. The EBC collection was made following the recommendations of the American Thoracic Society/European Respiratory Society Task Force on EBC (32). After collection, EBC samples were deaerated with inert argon gas (350 ml/min for 10 min), and pH was measured using a pH meter with a resolution of 0.01 and a working range from -2.00 to 16.00. The pH meter was calibrated using pH buffer solutions daily. Samples were then aliquoted in labeled crytotubes and an antioxidant mixture (butylated hydroxytoluene, 2 mM in 99% ethanol, 10 μl per ml sample) was added. Samples were immediately stored at -80°C for later analyses of the following biomarkers. The samples were analyzed for nitrite and nitrate using an HPLC-UV system at 214 nm (Waters, Milford, MA). The detection limits were 7.22 ng/ml for nitrite and 4.43 ng/ml for nitrate. Finally, the samples were analyzed for 8-isoprostane using an ELISA assay with a method detection limit of 1.56 pg/ml (Rapidbio, West Hills, CA).

We measured urinary 8-OHdG using an HPLC equipped with an electrochemical detector (Waters, Milford, MA) (33). The method detection limit was 0.5 ng/ml. Concentrations of urinary 8-OHdG were normalized by urinary creatinine concentrations and reported in mg/mol.

Air Pollution and Weather Measurements

We measured 24-hour mean concentrations of fine particles (particulate matter $[PM]_{2.5}$) and several of its constituents: elemental carbon (EC), organic carbon (OC), and sulfate (SO_4^{2-}) (30). We also measured 24-hour mean concentrations of sulfur dioxide (SO_2), nitrogen dioxide (NO_2), ozone (O_3), carbon monoxide (CO), and ambient temperature and relative humidity (RH). All these measurements were made on the roof top of a seven-story building in the center of the hospital campus. The methods have been described in more detail previously (29).

Statistical Analyses

We calculated period-specific descriptive statistics for each air pollutant, meteorologic variable, and biomarker by adding indicator variables for period (i.e., pre-, during-, and post-Olympics) into statistical models. Specifically, we used time-series regression models to estimate the period-specific means and between-period difference in air pollutant concentrations and meteorologic variables. We log-transformed F_{ENO}, nitrite, nitrate, nitrite plus nitrate, and 8-OHdG because these biomarkers had right-skewed distributions. We estimated changes in biomarker levels across the periods (pre-, during-, and post-Olympic period) using linear mixed models for all the biomarkers except 8-isoprostane. Because there were a substantial fraction of data below the detection limit for 8-isoprostane, we did not perform this analysis. In this set of models, we used each biomarker as the dependent variable and period as the independent variable.

We then used the next set of linear mixed models to estimate changes in biomarker concentration associated with each interquartile range (IQR) increase in pollutant concentration in the 24 hours before the clinic visit (lag day 0), and the six previous 24-hour periods (lags 1–6, respectively). This was done for all the biomarkers, except 8-isoprostane, each of which was assessed as a continuous variable. We dichotomized 8-isoprostane data into above (and equal to) and below 75th percentile (6.21 pg/ml) and used random-effects logistic regression models to assess odds ratios for getting a "higher" ($\geq$75th percentile) value associated with one IQR increase in pollutant concentration.

In all the models, we used the Akaike Information Criterion (AIC) to assess correlations between repeatedly measured data using the equicorrelated structure because this was the best structure consistently chosen across biomarkers among alternative correlation structures. Temperature, RH, sex, and day of week of clinic visits were controlled as covariates. Temperature and RH measured in the 24 hours before a visit were included in the models using natural splines with degrees of freedom chosen to minimize the AIC. We also accounted for additional seasonal differences by including 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 three degrees of freedom resulted in overfitting, we only allowed up to three degrees of freedom for all splines.

We examined adjusted associations between biomarker levels and pollutant concentrations averaged over the last 24 hours (lag 0), 24–48 hours (lag 1), 48–72 hours (lag 2) and so on, up to a 7-day lag. For all pollutant-biomarker combinations, "lag plots" were created to show the changes in biomarker level associated with one IQR increase in pollutant for lag 0 through lag 6. These were a series of single-lag models. Because some pollutants were correlated, we conducted two-pollutant models to examine whether the biomarker–pollutant associations from the single pollutant analyses were consistent with the effect of that pollutant while controlling for another pollutant. In these two-pollutant models, we only included one lag for each pollutant that showed the strongest statistical significance.

We conducted three sets of sensitivity analyses to evaluate the robustness of our models with respect to the adjustment of temperature and RH: (*1*) without any adjustments; (*2*) with full adjustments as described previously (our main analysis); and (*3*) with adjustments only when temperature or RH was significantly associated with biomarker in the main model. Furthermore, we examined potential confounding effects of seasonality by excluding post-Olympic period observations, and other time varying factors across periods by including period indicators in the single pollutant analyses.

We used R Version 2.12.1 (available on CRAN at http://cran.r-project.org) to perform all the analyses.

RESULTS

Descriptive statistics of air pollutant concentrations and meteorologic variables are summarized in Table 1. Across the entire study period, we observed large day-to-day variations in pollutant concentrations, as reflected in large IQR for each pollutant. From the pre-Olympic to the during-Olympic period, we observed significant decreases in mean concentrations of SO_2 (-60%), CO (-48%), NO_2 (-43%), and EC (-36%) and nonsignificant reductions in mean concentrations of $PM_{2.5}$ (-27%), SO_4^2 (-13%), and OC (-22%). The mean O_3 concentration and mean temperature increased from the pre-Olympic to the during-Olympic period. In contrast, from the during-Olympic to the post-Olympic period, we observed significant increases in pollutant concentrations for all the pollutants except O_3 and SO_4^2. Throughout the study period, several pairs of pollutants had correlation coefficients greater than 0.7: $PM_{2.5}$ and SO_4^2; EC and OC; EC and NO_2; OC and NO_2; OC and SO_2; and $PM_{2.5}$ and SO_2. O_3 was negatively correlated with NO_2, EC, OC, and CO, but was weakly correlated with SO_2, $PM_{2.5}$, and SO_4^2 ($r < 0.35$) (29).

TABLE 1. PERIOD-SPECIFIC MEANS AND SE FOR AIR POLLUTANTS, AMBIENT TEMPERATURE, AND RH AND PERIOD COMPARISON RESULTS

Pollutant/Meteorologic Parameter	IQR	Pre-Olympics (*June 2 to July 7*)		During-Olympics (*July 28 to August 29*)		Post-Olympics (*September 29 to October 31*)		Period Comparison			
								Difference (*during–pre*)		Difference (*post–during*)	
		Mean	SE	Mean	SE	Mean	SE	Mean	95% CI	Mean	95% CI
SO_2, ppb	5.4	7.45	1.17	2.97	1.33	6.81	1.22	−4.48*	(−7.94 to −1.02)	−0.64	(−3.95 to 2.67)
NO_2, ppb	18.7	25.60	3.66	14.61	3.76	41.39	3.81	−10.99*	(−21.26 to −0.71)	15.79*	(5.44 to 26.14)
O_3, ppb	25.4	31.84	3.75	39.60	3.85	15.12	3.91	7.75	(−2.78 to 18.29)	−16.73	(−27.34 to −6.11)
CO, ppm	0.65	1.23	0.13	0.64	0.14	0.81	0.14	−0.59*	(−0.97 to −0.22)	−0.42*	(−0.80 to −0.04)
$PM_{2.5}$, μg/m^3	76.8	98.9	14.7	71.9	15.1	85.3	15.3	−27	(−68.3 to 14.3)	−13.7	(−55.3 to 27.9)
EC, μg/m^3	1.4	2.2	0.3	1.4	0.3	3.4	0.3	−0.80	(−1.7 to 0.1)	1.1*	(0.3 to 2)
OC, μg/m^3	5.1	8.8	1.6	6.8	1.7	15	1.7	−1.97	(−6.6 to 2.6)	6.2*	(1.7 to 10.7)
Sulfate, μg/m^3	28	26.5	5.8	23	6.4	13.7	6.2	−3.5	(−20.4 to 13.5)	−12.8	(−29.5 to 3.9)
Temp, °C		25.2	0.9	27.8	0.9	16.7	0.9	2.52	(0.02 to 5.02)	−8.56†	(−11.08 to −6.04)
RH, %		65.6	4	64.9	4.1	49.5	4.2	−0.69	(−11.93 to 10.54)	−16.10*	(−27.42 to −4.78)

Definition of abbreviations: CI = confidence interval; IQR = interquartile range; RH = relative humidity; T = temperature.
The results were derived from time-series regression models.
* $P < 0.05$.
† $P < 0.01$.

Changes in Biomarker Levels by Period

Period-specific means and SE of the biomarkers, accounting for the repeated measures, are shown in Table 2. From the pre-Olympic to the during-Olympic period, we observed decreases in mean concentrations of F_{ENO}, nitrate, nitrite, nitrate plus nitrite, and 8-OHdG, respectively. We also observed an increase in EBC pH and a decrease in the fraction of above-detection-limit for EBC 8-isoprostane. These changes were reversed (increase vs. decrease) for all the biomarkers except nitrite from the during-Olympic to the post-Olympic period.

After adjusting for ambient temperature, RH, sex, and day of week for measurement, pre-Olympic to during-Olympic changes in biomarker levels were statistically significant for all the biomarkers in the hypothesized direction. As shown in Figure 2a, we observed large and significant decreases, ranging from −72.5 to −4.5%, in F_{ENO}, nitrite, nitrate, nitrite plus nitrate, and 8-OHdG, respectively. We also observed a significant increase in EBC pH (3.5% with 95% confidence interval, 2.2–4.9%), which corresponds to a large decrease (−46%) in EBC hydrogen ion. Likewise, during-Olympic to post-Olympic changes after adjusting for the covariates were similar to the results from unadjusted analysis shown in Table 2 (EBC nitrite change became statistically significant in the adjusted analysis). As shown in Figure 2b, we observed large increases, ranging from 48–362%, in F_{ENO}, nitrite, nitrate, nitrite plus nitrate, and 8-OHdG, respectively. We also observed a decrease in EBC pH (−4.8% with 95% confidence interval, −9.4 to −0.2%) corresponding to a large increase (146%) in EBC hydrogen ion concentration.

Biomarker–Pollutant Associations

As hypothesized, we observed significant increases in F_{ENO} (ranging from 10–75%) associated with IQR increases in $PM_{2.5}$, SO_4^{2-}, EC, SO_2, CO, and NO_2 at most lags (Figure 3a). The F_{ENO} changes per IQR increases (effect estimates) in most pollutants were largest at lag 0 (0–24 h before biomarker measurement), except SO_2, which had the largest effect estimate at lag 3. The effect estimates generally decreased with each increasing lag. However, for O_3, the direction of the association was negative at lags 0–2.

Consistent with the hypothesis, EBC nitrite was positively associated with all pollutants except O_3 at one or more lags (Figure 3b). Across the lags, 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, 20.5% for SO_2, 15.7% for CO, and 22.2% for NO_2. Pollutant associations of EBC nitrate varied more substantially across lags than those of EBC nitrite, in terms of variation directions and effect size (Figure 3c). As expected (Figure 3d), the pattern for nitrite plus nitrate was between that for nitrite and that for nitrate. The effect estimates were positive, significant, and largest for $PM_{2.5}$, SO_4^{2-}, EC, OC, and SO_2 at lag 0, and CO and NO_2 at lag 1. However, the association with O_3 was significant and negative at lags 0–2.

Consistent with the hypothesized mechanism that air pollution exposure leads to airway acidification, we observed significant negative associations between EBC pH and all the pollutants at most lags except O_3 at lags 0 and 6 (Figure 3e). 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 2 for SO_2; lags 0 and 5 for CO; lag 5 for NO_2; and lag 0 for O_3 (at the opposite direction).

TABLE 2. PERIOD-SPECIFIC MEANS AND SE FOR PULMONARY INFLAMMATION AND OXIDATIVE STRESS BIOMARKERS

Biomarkers	Pre-Olympics		During-Olympics		Post-Olympics	
	Mean	SE	Mean	SE	Mean	SE
F_{ENO}, ppb*	11.76	1.04	5.80	1.04	12.51	1.04
EBC nitrite, μM*	7.33	1.03	4.71	1.03	4.69	1.04
EBC nitrate, μM*	2.79	1.04	2.61	1.04	4.23	1.05
EBC nitrite + nitrate, μM*	10.48	1.03	7.68	1.03	10.37	1.03
EBC pH	7.43	0.03	7.46	0.03	7.61	0.03
EBC 8-isoprostane, % ⩾DL 1.56 pg/ml	68	N/A	44	N/A	74	N/A
Urinary 8-OHdG, mg/mol*	3.70	1.12	2.22	1.12	3.34	1.12

Definition of abbreviations: DL= detection limit; EBC = exhaled breath condensate; F_{ENO} = fractional exhaled nitric oxide; 8-OHdG = 8-hydroxy-2-deoxyguanosine.
The results are based on period estimates from mixed-effects models, accounting for the repeated measures but not adjusting for covariates.
* Biomarkers had skewed data distributions, for which geometric means were reported.

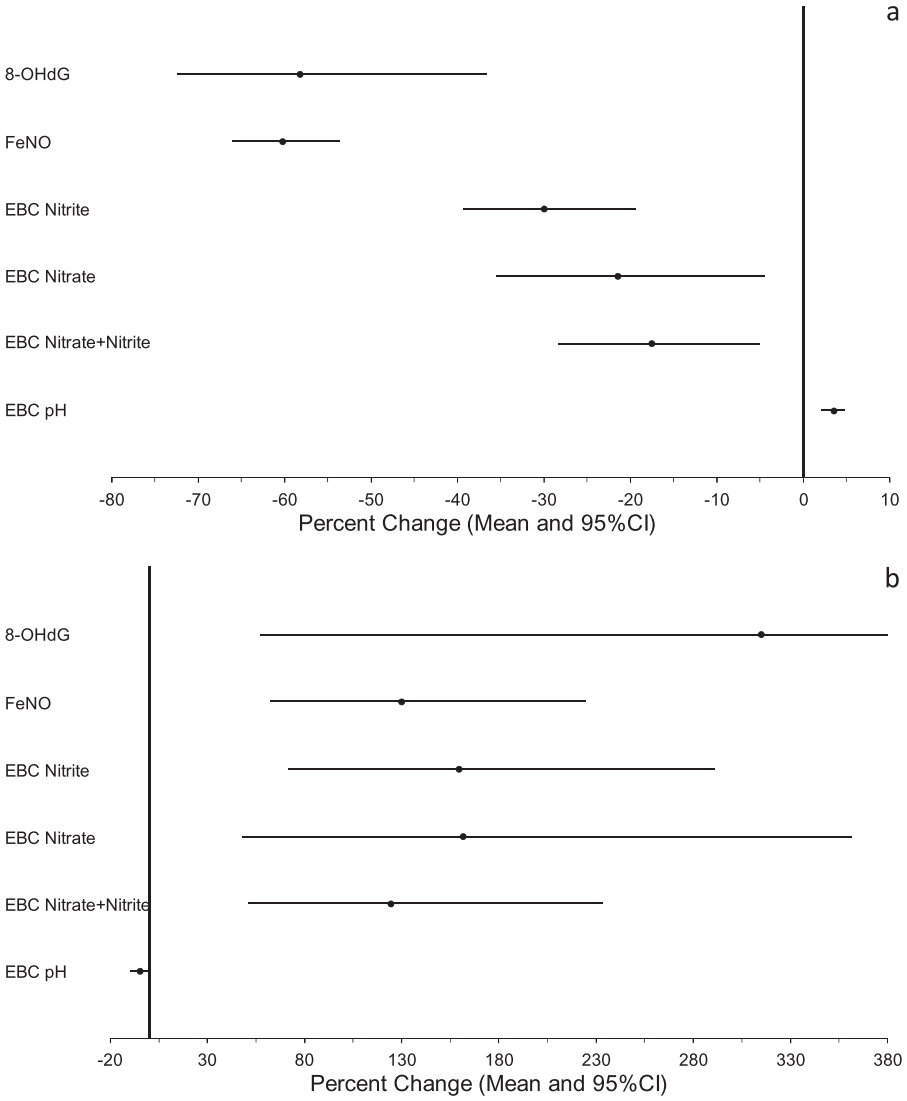


Figure 2. Point estimates and 95% confidence interval (CI) of percent changes in biomarker levels (*a*) from pre-Olympic to during-Olympic period, and (*b*) from during-Olympic to post-Olympic period. Estimates were derived from linear mixed-model analysis controlling for ambient temperature, relative humidity, sex, and day of week of measurement. EBC = exhaled breath condensate; F_{ENO} = fractional exhaled nitric oxide; 8-OHdG = 8-hydroxy-2-deoxyguanosine.

Consistent with the hypothesis that increased exposure to air pollution leads to increased lipid peroxidation in the lungs, we observed an increased odds ratio of a high EBC 8-isoprostane level (⩾75th percentile relative to <75th percentile values) associated with each IQR increase in all the pollutant except O_3 at multiple lags (Figure 3f). The increased odds ratios were statistically significant for $PM_{2.5}$ at lags 3 and 4; SO_4^{2-} at lag days 4 and 5; EC at lag day 4; OC at lag 4; SO_2 at lag days 0, 3, and 4; CO at lag 4; and O_3 at lag 4 (but in the opposite direction).

As shown in Figure 3g for urinary 8-OHdG, we observed mostly positive and significant associations for all pollutants (except O_3) across multiple lags, which is consistent with the hypothesis that increased air pollution exposure leads to increased whole-body burden of oxidative stress. The largest changes in 8-OHdG concentration per IQR increase in pollutant concentration were 57.6% for $PM_{2.5}$, 75.4% for sulfate, 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). The largest change in 8-OHdG level associated with an IQR increase in O_3 concentration was −30.5% (opposite direction) at lag 5.

In two-pollutant model analyses, when adjusting for a second pollutant in the same model, we observed small changes in the individual pollutant-biomarker effect estimates for most of the biomarkers, compared with those in the single-pollutant model analysis. However, no substantial changes were observed in the overall pattern of the associations (*see* Figure E1 in the online supplement).

Sensitivity Analyses

Table E1 provides degrees of freedom selected for each meteorologic parameter in the statistical analysis. When nonsignificant temperature and RH were deleted from the models or without controlling for temperature and RH, the changes in the between-period and the overall pattern in the pollutant-biomarker associations remained similar to those observed in the main model (primary) analyses (*see* Figure E2).

Results from single-pollutant models excluding the post-Olympic observations for selected biomarkers (F_{ENO}, EBC nitrite, and urinary 8-OHdG) are summarized in Figure E3. As expected, the reduced sample size in the two-period-only analysis resulted in wider confidence intervals of the effect estimates but did not change the main findings. To assess within-period effects of the single pollutants, we added the "period" indicator as a covariate to the single-pollutant models. Results from this set of analysis are shown in Figure E4 for selected biomarkers. We observed, in general, more conservative (reduced) effect estimates of the pollutant-biomarker associations compared with the effect estimates when "period" was not adjusted, except for EBC nitrite–nitrate, for which this difference was less notable.

Findings from these sensitivity analyses further support our main findings, indicating that changes in biomarker levels between periods and in association with pollutant concentrations were unlikely driven by other time-varying factors than changes in pollution levels.

DISCUSSION

Taking advantage of a unique experimental opportunity where the Chinese government mandated temporary closures or relocations of industry and reductions in motor vehicle use during the Beijing Olympics, we observed significant changes in multiple biomarkers in healthy and young adults in response to drastic changes in air pollution levels. We also observed significant associations of each of these biomarkers with multiple pollutants after adjusting for meteorologic parameters. The biomarkers we used in the study, reflecting pulmonary inflammation, respiratory, and whole-body oxidative stress, were sensitive to changes in air pollution levels across the three periods of the quasi-experimental design and in the day-to-day biomarker–pollutant association analysis. This demonstrates the use of the novel and noninvasive biomarkers in examining airway inflammation and oxidative stress attributable to ambient pollution even in healthy and young adults.

F_{ENO} has been widely used as a sensitive biomarker of airway inflammation (22, 34, 35). Increases in F_{ENO} concentration have been associated with increases in air pollution levels in children and adults with asthma (13, 15, 19, 36). Our study further confirms the F_{ENO} and air pollution association in healthy and young adults. Lowering of the airway pH has been reported to cause bronchoconstriction (37); to impair airway epithelial functions, such as ciliary motility (38); and to increase airway mucus viscosity (39). Decreases in EBC pH in patients with asthma suggests that acute asthma exacerbations may be accompanied by airway acidification that reflects inhibition of local epithelial proton pumps during airway inflammation (16). Our study is perhaps the first to examine and confirm air pollution associations with airway acidification in healthy adults.

Much of the effort to identify a biochemical basis for acute and chronic effects of air pollution has centered on the role of reactive oxygen species (ROS) induced from inhaled air pollutants, especially PM (10). These ROS may result in an increased burden of oxidative stress and proinflammatory effects, locally and distant from the site of initial injury (10, 40–42). EBC nitrite and nitrate (or the sum: nitrite plus nitrate) are metabolic oxidation products of NO that is produced primarily in the lung. Concentrations of nitrite and nitrate in EBC, hence, reflect levels of oxidative and nitrosative stress in the respiratory tract (43, 44). ROS can react with lipids to form stable compounds, such as 8-isoprotane. Increased EBC concentrations of nitrite and nitrate and 8-isoprostane have been associated with exacerbation of asthma, chronic obstructive pulmonary disease, and cystic fibrosis (45–49). However, to the best of our knowledge, there have been no published studies reporting EBC nitrite and nitrate changes in relation to air pollution exposure. Only two studies have reported increases in EBC 8-isoprostane concentration associated with air pollution exposure: one in children with asthma and one in patients with coronary heart disease (12, 17). In the present study, we observed significant increases in EBC nitrite, nitrate, and nitrite plus nitrate, each associated with increased concentrations of $PM_{2.5}$, EC, OC, and sulfate with largest effects at lag 0. In contrast, EBC 8-isoprostane showed its largest effect estimates at lag 3 or lag 4 for $PM_{2.5}$, sulfate, SO_2, and CO. This suggests that air pollution may affect NO metabolism faster than it induces lipid peroxidation.

ROS can oxidize DNA molecules to form stable products (e.g, 8-OHdG) that are readily excreted in biofluids, such as urine. Increased 8-OHdG levels have been positively associated with premature coronary heart disease mortality (50) and with coronary artery disease but negatively associated with total serum antioxidant capacity (51, 52). In the present study, we observed a large (58%) and significant reduction in urinary 8-OHdG concentration from the pre- to the during-Olympic period and a very large (315%) reversal from the during-Olympic to the post-Olympic period. This finding supports an association between lowered whole-body oxidative stress and improved air quality. Although there has been a considerable literature reporting associations between ambient PM and oxidative stress in children and adults with asthma or other cardiorespiratory diseases (20, 36, 53–55), our study provides evidence in healthy and young adults, with both respiratory tract and whole-body biomarkers, to support the oxidative stress mechanism.

We also reported previously that biomarkers of systemic inflammation and thrombosis were significantly associated with changes in air pollution levels during the Beijing Olympics (29). This suggests a potential interactive relationship between the respiratory pathway markers demonstrated in the present data and the cardiovascular markers previously reported, all potentially contributing to clinical cardiovascular endpoints. 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 preexisting atherosclerotic plaques or activation of platelets. Thrombosis, dependent on platelet activation, is responsible for arterial obstruction on top of such a ruptured plaque and ultimately an ischemic myocardial or cerebral event, as postulated previously (8).

Strengths and Limitations

Most previous studies exploring underlying biologic mechanisms of air pollution health effects have focused on relationships among a few biomarkers and air pollutants based on hourly, daily, or weekly variations in pollutant levels. In this study, we measured a suite of exhaled breath biomarkers of respiratory inflammation and oxidative and nitrosative stress, some of which (e.g., EBC pH, nitrite, and nitrate) are novel and have seldom been used in air pollution studies. Furthermore, we observed significant associations, in the hypothesized direction, between biomarker levels and pollutant concentrations in a homogenous group of young and healthy individuals, without potential confounding from disease, medication use, and age-related susceptibility.

However, several limitations should be noted for our study. First, although our pre-Olympic and during-Olympic measurements all occurred in summer months, the post-Olympic measurements fell in the early autumn season. We conducted a series of sensitivity analyses using different methods to adjust for temperature and RH (e.g., no adjustment, linear or natural splines adjustments with different degrees of freedom). The sensitivity analysis results indeed showed some impact on a few biomarkers (e.g., EBC nitrite) in terms of statistical significance. However, the overall lag pattern of the associations and effect estimates remained stable. Second, our measurements of air pollution were made at a fixed site near the work and residence places of the subjects and were not personal exposure measurements. Although we were not able to conduct a validation study to examine personal exposure in relation to outdoor pollution with portable samplers, we anticipated that indoor microenvironmental exposure would be highly influenced by the outdoor air because the infiltration rate was generally high in buildings during the summer and early fall in Beijing. Third, it is known that some of the biomarkers may be affected by diet. For example, a nitrate-rich diet may increase F_{ENO} levels (and likely EBC nitrate and nitrite levels) (56, 57). However, it is unlikely that our study subjects (medical professionals) would have changed their diet during the Olympics. Hence, observed changes in biomarkers during the Olympics compared with before and after Olympics are unlikely to have resulted from dietary changes and other behavioral changes. Last, we attempted to address what

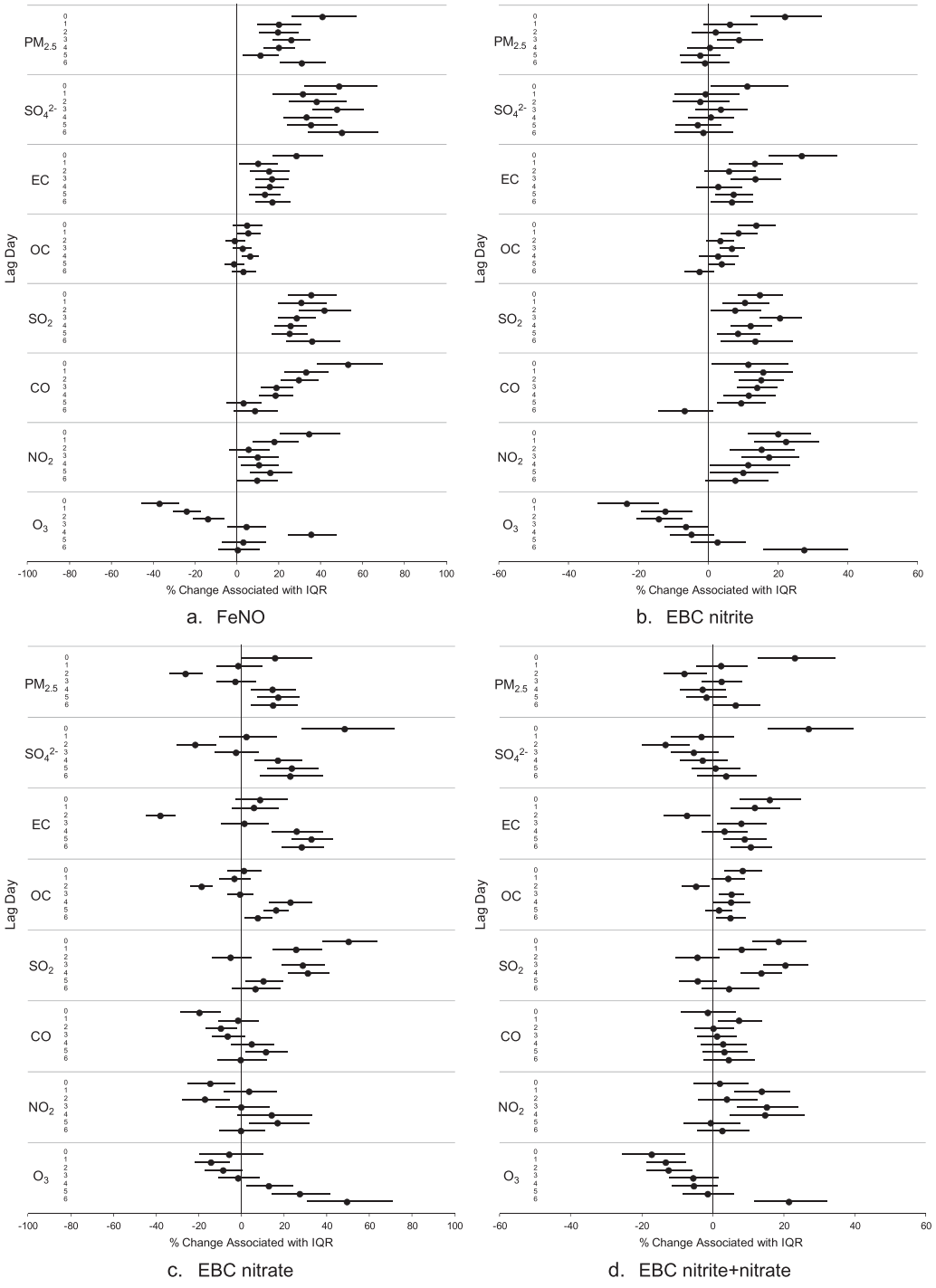
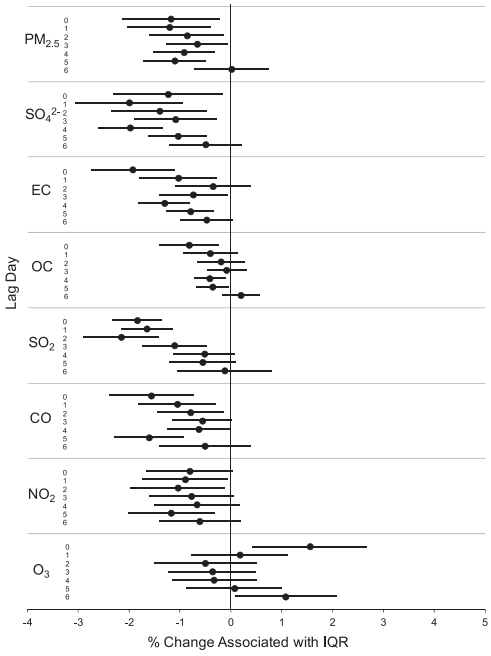


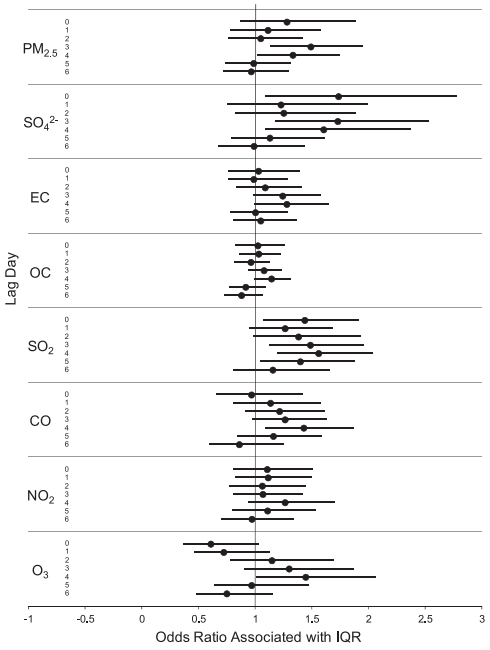
Figure 3. Percent changes and 95% confidence intervals in biomarker levels (odds ratios for having a >75th percentile value of exhaled breath condensate [EBC] 8-isoprostane) associated with one interquartile range (IQR) increase in pollutant concentration, by 24-hour lag period (lag Day 0–6), for the whole study period. (*a*) Fractional exhaled nitric oxide (Fe_{NO}). (*b*) EBC nitrite. (*c*) EBC nitrate. (*d*) EBC nitrite and nitrate. (*e*) EBC pH. (*f*) EBC 8-isoprostane. (*g*) 8-hydroxy-2-deoxyguanosine (8-OHdG).

specific components of the air pollution mixture (e.g., gases versus PM) and what specific constituents of PM were more "responsible" for changes in biomarker levels. However, in part because of the simultaneous shut-down of multiple pollutant sources during the Olympics and subsequent relaxation of pollution controls, it was difficult to differentiate effects of specific pollutants, particularly when many pollutants were correlated among each other. The seemingly "beneficial" effects of ozone on several biomarkers was likely caused by the fact that O_3 concentrations were increased, whereas the other pollutants were substantially reduced during the Olympic air pollution control period (29). A similar seemingly protective effect of O_3 has also been observed in previous studies when O_3 was negatively correlated to NO_2 and other pollutants (58, 59).

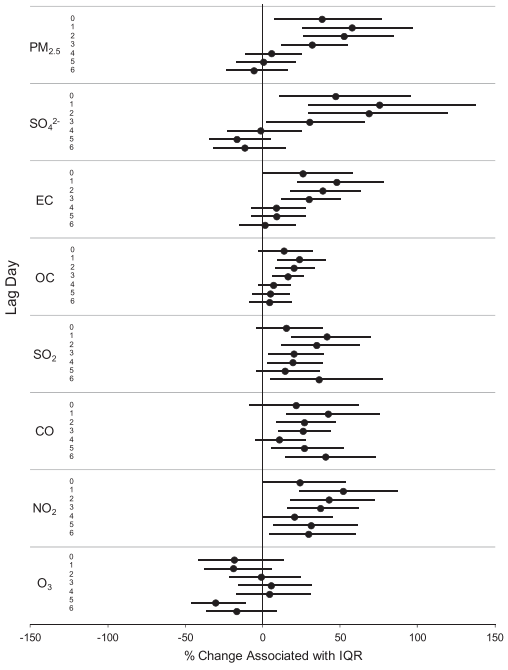
It is important to recognize some fundamental limitations of the EBC approach (32, 60). The most notable one is perhaps the lack of the standardization of EBC biomarker concentrations because of variable dilution during EBC collection. To minimize the variability in dilution, we collected EBC samples following the recommendation by the American Thoracic Society/European Respiratory Society Task Force on EBC (32), although we realize this cannot completely eliminate this problem. Another recognized major limitation is potential contamination of EBC biomarkers from oral microorganisms. These limitations have largely prevented the use of EBC biomarkers in clinical settings. However, in a repeated within-person comparison design like the present study, dilution and oral contamination would introduce random errors, but



e. EBC pH



f. EBC 8-isoprostane



g. 8-OHdG

Figure 3. (*Continued*).

cannot produce the consistent relationships between the multiple EBC biomarker levels and the periods or the pollutant concentrations.

In summary, we observed significant improvements in biomarkers of pulmonary inflammation and biomarkers of respiratory and systemic oxidative stress during the Beijing Olympic air pollution control period. We also observed significant increases in levels of pulmonary inflammation, respiratory tract oxidative stress, and systemic oxidative stress, associated with increases in pollutant concentrations measured 0–24 hours to 6 days before biomarker measurements. The largest adverse biomarker changes per IQR increase in pollutant concentration (effect estimates) were seen within 24 or 48 hours for biomarkers of pulmonary inflammation (FE_{NO}); airway acidification (EBC pH); and metabolic products of NO (EBC nitrite, nitrate, and nitrite plus nitrate). The largest effect estimates were observed slightly later (48–96 h) for biomarkers of lipid preoxidation (EBC 8-isoprostane) and DNA oxidative damage. These findings support the hypothesis that air pollution adversely affects health through acute changes in pulmonary inflammation and oxidative damage to essential biomolecules, such as lipids and DNA. The findings also demonstrate the potential utility of novel and noninvasive biomarkers used in the present study to examine biologic mechanisms of pulmonary effects in the general population consisting largely of healthy adults.

Author disclosures are available with the text of this article at www.atsjournals.org.

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