

## Supplemental Material

### Short-term Exposure to Ambient Air Pollution and Biomarkers of Systemic Inflammation: The Framingham Heart Study

Wenyuan Li, SD<sup>1,2</sup>, Kirsten S. Dorans, SD<sup>1,2</sup>, Elissa H. Wilker, ScD<sup>2,3</sup>, Mary B. Rice, MD, MPH<sup>4</sup>, Petter L. Ljungman, MD, PhD<sup>2,5</sup>, Joel D. Schwartz, PhD<sup>1,3</sup>, Brent A. Coull, PhD<sup>6</sup>, Petros Koutrakis, PhD<sup>3</sup>, Diane R. Gold, MD, MPH<sup>3,7</sup>, John F. Keaney, Jr, MD<sup>8</sup>; Ramachandran S. Vasani, MD<sup>9,10,11</sup>, Emelia J. Benjamin, MD, ScM<sup>9,10,11</sup>, Murray A. Mittleman, MD, Dr.PH<sup>1,2</sup>

<sup>1</sup>Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA

<sup>2</sup>Cardiovascular Epidemiology Research Unit, Division of Cardiology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA

<sup>3</sup>Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA

<sup>4</sup>Division of Pulmonary, Critical Care and Sleep Medicine, Beth Israel Deaconess Medical Center, Boston, MA

<sup>5</sup>Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

<sup>6</sup>Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA

<sup>7</sup>Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

<sup>8</sup>Division of Cardiovascular Medicine, University of Massachusetts Medical School, Worcester, MA

<sup>9</sup>National Heart, Lung, and Blood Institute's and Boston University's Framingham Heart Study, Framingham, MA

<sup>10</sup>Preventive Medicine and Cardiovascular Medicine Sections, Department of Medicine, Boston University School of Medicine, Boston, MA

<sup>11</sup>Department of Epidemiology, Boston University School of Public Health, Boston, MA

## Materials and Methods

### Study Sample

We included participants from the Framingham Offspring cohort examination cycle 7 (1998-2001), cycle 8 (2005-2008), Third Generation cohort examination cycle 1 (2002-2005), and cycle 2 (2008-2011). The study design and selection criteria of the two cohorts have been described elsewhere.<sup>1, 2</sup> First, we restricted our analyses to 4,110 participants who lived within 50 km from the Harvard Supersite air pollution monitor in Boston, Massachusetts and who were not current smokers at the time of their examination visit. We excluded current smokers (1,139 observations) because levels of inflammatory biomarkers were likely already elevated among current smokers and may affect our ability to assess the relatively small variation in levels of inflammatory biomarkers that could be attributable to ambient air pollution,<sup>3</sup> similar to our previous work.<sup>4</sup> Then we assigned “missing status” to biomarker levels that were under the minimum detection limit (27 observations for CRP, 10 observations for interleukin-6, and 1 observation for TNFR2) and 1 TNFR2 measurement that had an extremely high value (948,114 pg/ml). Last, we excluded 65 participants who had no measurement of any biomarkers and 49 participants who had missing information on covariates including body mass index, alcohol intake, or pack years of smoking, leaving 3,996 participants in the final analytic dataset. At each examination visit, physical examinations were performed following standardized protocols, and data on demographics, medication history, smoking history, and alcohol intake were collected using standardized questionnaires. All participants provided written informed consent at each examination, and the Institutional Review Boards at Beth Israel Deaconess Medical Center and Boston University Medical Center approved the study.

### Air Pollution Assessment

Previous studies have shown that the blood levels of the inflammatory biomarkers could increase within a few days after inflammation,<sup>5, 6</sup> thus we calculated 1-, 2-, 3-, 5-, and 7-day moving averages of the air pollutants prior to the date of examination visit, based on hourly measures of PM<sub>2.5</sub>, black carbon (BC), and sulfate (SO<sub>4</sub><sup>2-</sup>) from the Boston Harvard Supersite air pollution monitoring station, and measures of nitrogen oxides (NO<sub>x</sub>) and ozone (O<sub>3</sub>) from local state monitors within the Greater Boston area.<sup>7</sup> The central monitor is located on the rooftop of the Francis A. Countway Library of Medicine (5 stories above ground level) and 50 m from the nearest street. PM<sub>2.5</sub> was measured using a tapered element oscillating microbalance (Model 1400A, Rupprecht & Patashnick Co., Inc.); BC was measured using an aethalometer (Model AE16, Magee Scientific Corp.). We calculated daily average SO<sub>4</sub><sup>2-</sup> from elemental sulfur that was measured by X-Ray Fluorescence analysis of the PM<sub>2.5</sub> filter samples, and used an SO<sub>4</sub><sup>2-</sup> analyzer (Model 5020, Thermo Electron Corp.) on days when SO<sub>4</sub><sup>2-</sup> X-Ray Fluorescence data were not available. The detailed measurement methods have been described previously.<sup>8</sup> We also obtained hourly temperature and relative humidity data from the Boston Logan International Airport Weather Station, located 12 km from the central site.<sup>7</sup>

### Biomarker Assessment

Blood samples were collected after an overnight fast and stored at  $-80^{\circ}\text{C}$  until assayed. Plasma samples were analyzed to measure  $\text{TNF}\alpha$ ,  $\text{TNFR2}$ , and fibrinogen, and serum samples were used for CRP and IL-6. CRP was measured twice in each cohort (Offspring examination 7 and Third Generation examination 1: immunonephelometry, Dade Behring BN 100 nephelometer; Offspring examination 8 and Third Generation examination 2: immuno-turbidometry, R&D Systems). Fibrinogen was measured using the Clauss method (Diagnostica Stago Reagents) in Offspring examination 7 and Third Generation examination 1.  $\text{TNFR2}$  was measured twice in the Offspring cohort and once in the Third generation cohort, Interleukin-6 was measured in Offspring examination 8 and Third Generation examination 1, and  $\text{TNF}\alpha$  was measured in Offspring examination 7, all by commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems). Details regarding biomarker measurements have been reported elsewhere<sup>9-11</sup> and can be found at the Framingham Heart Study website: <https://www.framinghamheartstudy.org/researchers/description-data/noninvasive-biomarker.php>.

### Statistical Methods

We fit multivariable linear regression models for fibrinogen, interleukin-6, and  $\text{TNF}\alpha$ , and multivariable linear mixed effects models with participant-specific random intercepts for CRP and  $\text{TNFR2}$  for each of the 1-, 2-, 3-, 5-, and 7-day moving averages before the date of examination visit. Levels of the biomarkers were  $\log_e$  transformed to approximate a normal distribution. In each model, we adjusted for demographic and socio-economic position variables including centered age at the time of examination visit, (centered age)<sup>2</sup>, sex, educational attainment (high school or less, some college, and college graduate), census tract-level median household income (continuous); lifestyle variables including body mass index, alcohol intake (drinks/week; continuous, standardized to 0.5 oz [15 ml] alcohol/drink),<sup>12</sup> pack years of smoking (continuous), and smoking status (never or former smoker); and an examination identifier (categorical). All census data were from U.S. Census 2000. We adjusted for seasonality and time by adding sine and cosine terms of the examination date and a linear term of the examination date, and adjusted for meteorology by adding corresponding moving averages of temperature and relative humidity.

For sensitivity analyses, we examined whether the associations differed if we: 1) excluded observations that had a daily average  $\text{PM}_{2.5}$  concentration  $>35 \mu\text{g}/\text{m}^3$  (U.S. Environmental Protection Agency 24-hour  $\text{PM}_{2.5}$  standard) in any one of the 7 days prior to the examination date; 2) included current smokers in the analyses; 3) restricted study participants to those who lived within 40 km from the central monitoring site; and 4) additionally adjusted for usual occupation (laborer; sales/homemaker/clerical; professional/executive/supervisory/technical; and unspecified),<sup>13</sup> census tract median value of owner occupied housing units (continuous), census tract population density (people/ $\text{km}^2$ , continuous), physical activity index (in tertiles),<sup>14</sup> cardiovascular disease status, antihypertensives use, and statins use. We also explored whether associations differed by median age of the overall available study sample (53 years old), sex, education level (high school or less *versus* higher than high school), diabetes status, cardiovascular disease status, anti-hypertensives use, statins use, and season (warm [April to September] *versus* cold [October to March]) by adding an interaction term to

the models. Because we pooled participants from both cohorts together in the primary analyses, we examined the associations separately in each cohort. Further, because not all participants have data on all moving averages, we conducted a sensitivity analysis where we restricted analyses to the same participants across all moving averages for each pollutant. For all analyses, we created missing indicators for participants with missing data on educational attainment (35 observations) or physical activity (96 observations).

Parameter estimates from the analyses were scaled by a factor close to the interquartile range for the 1-day moving average of each pollutant: 5  $\mu\text{g}/\text{m}^3$  for  $\text{PM}_{2.5}$ , 0.5  $\mu\text{g}/\text{m}^3$  for BC, 2  $\mu\text{g}/\text{m}^3$  for  $\text{SO}_4^{2-}$ , 20 ppb for  $\text{NO}_x$ , and 10 ppb  $\text{O}_3$ . We reported estimated percent differences with 95% confidence intervals (CIs). As to interpreting the results from our primary analyses, we focused on describing the observed association patterns between pollutants and the biomarkers across moving averages and pollutants. For sensitivity analyses in which effect modification was examined, we used the two-tailed  $p$ -value from the Wald test of the interaction term to judge whether the associations differed between subgroups (considered statistically significant if  $p < 0.05$ ). We reported and highlighted association patterns that we considered consistent across moving averages or pollutants. Analyses were performed using PROC GENMOD and PROC MIXED in SAS 9.4 (SAS Institute, Inc., Cary, NC). Figures were plotted using Stata 13 (StataCorp LP, College Station, TX).

## References

1. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families. The framingham offspring study. *Am J Epidemiol.* 1979;110:281-290.
2. Splansky GL, Corey D, Yang Q, Atwood LD, Cupples LA, Benjamin EJ, D'Agostino RB, Sr., Fox CS, Larson MG, Murabito JM, O'Donnell CJ, Vasan RS, Wolf PA, Levy D. The third generation cohort of the national heart, lung, and blood institute's framingham heart study: Design, recruitment, and initial examination. *Am J Epidemiol.* 2007;165:1328-1335.
3. Levitzky YS, Guo CY, Rong J, Larson MG, Walter RE, Keaney JF, Jr., Sutherland PA, Vasan A, Lipinska I, Evans JC, Benjamin EJ. Relation of smoking status to a panel of inflammatory markers: The framingham offspring. *Atherosclerosis.* 2008;201:217-224.
4. Li W, Wilker EH, Dorans KS, Rice MB, Schwartz J, Coull BA, Koutrakis P, Gold DR, Keaney JF, Jr., Lin H, Vasan RS, Benjamin EJ, Mittleman MA. Short-term exposure to air pollution and biomarkers of oxidative stress: The framingham heart study. *J Am Heart Assoc.* 2016;5
5. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med.* 1999;340:448-454.
6. Oda S, Hirasawa H, Shiga H, Nakanishi K, Matsuda K, Nakamura M. Sequential measurement of il-6 blood levels in patients with systemic inflammatory response syndrome (sirs)/sepsis. *Cytokine.* 2005;29:169-175.

7. Ljungman PL, Wilker EH, Rice MB, Schwartz J, Gold DR, Koutrakis P, Vita JA, Mitchell GF, Vasani RS, Benjamin EJ, Mittleman MA, Hamburg NM. Short-term exposure to air pollution and digital vascular function. *Am J Epidemiol*. 2014;180:482-489.
8. Kang CM, Koutrakis P, Suh HH. Hourly measurements of fine particulate sulfate and carbon aerosols at the harvard-u.S. Environmental protection agency supersite in boston. *J Air Waste Manag Assoc*. 2010;60:1327-1334.
9. Pou KM, Massaro JM, Hoffmann U, Vasani RS, Maurovich-Horvat P, Larson MG, Keaney JF, Jr., Meigs JB, Lipinska I, Kathiresan S, Murabito JM, O'Donnell CJ, Benjamin EJ, Fox CS. Visceral and subcutaneous adipose tissue volumes are cross-sectionally related to markers of inflammation and oxidative stress: The framingham heart study. *Circulation*. 2007;116:1234-1241.
10. Fontes JD, Yamamoto JF, Larson MG, Wang N, Dallmeier D, Rienstra M, Schnabel RB, Vasani RS, Keaney JF, Jr., Benjamin EJ. Clinical correlates of change in inflammatory biomarkers: The framingham heart study. *Atherosclerosis*. 2013;228:217-223.
11. McManus DD, Beaulieu LM, Mick E, Tanriverdi K, Larson MG, Keaney JF, Jr., Benjamin EJ, Freedman JE. Relationship among circulating inflammatory proteins, platelet gene expression, and cardiovascular risk. *Arterioscler Thromb Vasc Biol*. 2013;33:2666-2673.
12. Elias PK, Elias MF, D'Agostino RB, Silbershatz H, Wolf PA. Alcohol consumption and cognitive performance in the framingham heart study. *Am J Epidemiol*. 1999;150:580-589.
13. Loucks EB, Lynch JW, Pilote L, Fuhrer R, Almeida ND, Richard H, Agha G, Murabito JM, Benjamin EJ. Life-course socioeconomic position and incidence of coronary heart disease: The framingham offspring study. *Am J Epidemiol*. 2009;169:829-836.
14. Kannel WB, Sorlie P. Some health benefits of physical activity. The framingham study. *Arch Intern Med*. 1979;139:857-861.