Ambient Particulate Air Pollution, Heart Rate Variability, and Blood Markers of Inflammation in a Panel of Elderly Subjects

C. Arden Pope III,1 Matthew L. Hansen,1,2 Russell W. Long,3,4 Karen R. Nielsen,5 Norman L. Eatough,3 William E. Wilson,6 and Delbert J. Eatough3

1Department of Economics, Brigham Young University, Provo, Utah, USA; 2Case Western Reserve University School of Medicine, Cleveland, Ohio, USA; 3Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah, USA; 4Human Exposure and Atmospheric Sciences, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA; 5Department of Radiation Oncology, University of Utah School of Medicine, Salt Lake City, Utah, USA; 6Office of Environmental Information, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA

Epidemiologic studies report associations between particulate air pollution and cardiopulmonary morbidity and mortality. Although the underlying pathophysiologic mechanisms remain unclear, it has been hypothesized that altered autonomic function and pulmonary/systemic inflammation may play a role. In this study we explored the effects of air pollution on autonomic function measured by changes in heart rate variability (HRV) and blood markers of inflammation in a panel of 88 elderly subjects from three communities along the Wasatch Front in Utah. Subjects participated in multiple sessions of 24-hr ambulatory electrocardiographic monitoring and blood tests. Regression analysis was used to evaluate associations between fine particulate matter (PM2.5) and HRV, C-reactive protein (CRP), blood cell counts, and whole blood viscosity. A 100-µg/m³ increase in PM2.5 was associated with approximately a 35 (SE = 8)-msec decline in standard deviation of normal R-R intervals (SDNN, a measure of overall HRV); a 42 (SE = 11)-msec decline in square root of the mean of the squared differences between adjacent normal R-R intervals (r-MSSD, an estimate of short-term components of HRV); and a 0.81 (SE = 0.17)-mg/dL increase in CRP. The PM2.5–HRV associations were reasonably consistent and statistically robust, but the CRP association dropped to 0.19 (SE = 0.10) after excluding the most influential subject. PM2.5 was not significantly associated with white or red blood cell counts, platelets, or whole-blood viscosity. Most short-term variability in temporal deviations of HRV and CRP was not explained by PM2.5; however, the small statistically significant associations that were observed suggest that exposure to PM2.5 may be one of multiple factors that influence HRV and CRP. Key words: cardiovascular disease, C-reactive protein, ECG monitoring, heart rate variability, inflammation, particulate air pollution, PM2.5.

Evidence is accumulating that particulate matter (PM) air pollution is associated with increased cardiopulmonary morbidity and mortality. [Committee of the Environmental and Occupational Health Assembly of the American Thoracic Society (CEOH-ATS) 1996; Pope and Dockery 1999; Pope et al. 2002]. Although the underlying physiologic mechanisms for these effects are still being explored, it has been postulated that PM’s influence could involve altered autonomic function and inflammatory responses indicated by various blood markers (Glantz 2002; Godleski et al. 2000; Seaton et al. 1995; Stone and Godleski 1999; Uretl et al. 2002). Epidemiologic studies have observed elevated PM exposures to be associated with specific physiologic end points, including reduced lung function (Hoek et al. 1998), increased blood plasma viscosity (Peters et al. 1997), reduced heart rate variability (HRV; Gold et al. 2000; Liao et al. 1999; Pope et al. 1999), and markers of inflammation (Ghio and Devlin 2001; Peters et al. 2001; Salvi et al. 1999, 2000; Schwartz 2001; Tan et al. 2000). It has also been suggested that certain groups may be more at risk for the effects of PM exposure, including the elderly (Pope 2000). In this study, we hypothesized that altered autonomic function and pulmonary inflammation play a role in the pathogenesis of cardiopulmonary disease related to fine particle air pollution [aerodynamic diameter ≤ 2.5 µm (PM2.5)]. A primary objective of this study was to further examine PM’s influence on cardiac autonomic function, as measured by HRV and blood markers of inflammation, by studying a panel of elderly persons who may be more susceptible or exhibit greater response to PM.

Materials and Methods

Study areas and periods. This study was conducted in three Utah communities: a) a community in Salt Lake City, Utah, referred to as Hawthorne, located 2.5 mi (4 km) southeast of the city’s urban center; b) Lindon, located in the Provo/Orem metropolitan area approximately 40 mi (65 km) south of Salt Lake City; and c) Bountiful, a Salt Lake City suburb, located approximately 12 mi (20 km) north of the urban center. Sources of PM in the communities included substantial traffic and urban-related sources, an integrated steel mill, and local oil refineries. All three communities are located along the Wasatch Front, a relatively densely populated area running north and south along the western front of the Wasatch Mountains. During winter low-level temperature inversion episodes, PM concentrations become elevated as local emissions are trapped in a stagnant air mass near the valley floor. Thus, PM pollution levels in the winter are generally higher and have much greater variability than during other seasons. In Hawthorne, data were collected during the winter of 1999/2000 and the summer of 2000. In Bountiful and Lindon, data were collected during the winter of 2000/2001.

Panel participants. Panels of elderly residents of the three communities were recruited to participate in 24-hr ambulatory electrocardiographic (ECG) monitoring and blood tests. Potential participants were initially recruited by directly contacting persons living in the neighborhoods adjacent to the monitoring sites and asking for neighborhood referrals. Information about the study was given, and for those who indicated a willingness to participate, an eligibility questionnaire was completed. A total of 89 persons were initially enrolled in the study. Six persons in Hawthorne who participated in the winter panel also participated in the subsequent summer panel and, for purposes of this analysis, were treated as separate subjects, which resulted in 95 subjects. Three subjects withdrew from the study before data collection began, and four more withdrew with only a single day of data collection, finally resulting in 88 subjects with 250 total observations (~2.84 observations per subject). All subjects were nonsmokers living in homes with no smokers.

Address correspondence to C.A. Pope III, 142 FOB, Brigham Young University, Provo, UT 84602 USA. Telephone: (801) 422-2157. Fax: (801) 422-0194. E-mail: cap3@email.byu.edu

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Subjects were retired persons between 54 and 89 years of age, and 57% were female. All subjects lived in private homes or were residents of a retirement home without special air filtration systems and had no serious medical conditions that would preclude their participation. Medical conditions that precluded participation included diabetes, renal failure, Parkinson’s disease, mental illness, chronic alcohol abuse, treatment with oxygen therapy, abnormal heart rhythm, pacemaker use, implanted defibrillator use, heart transplant, or heart failure within the previous 6 months. Research protocols and consent forms were approved by the institutional review board for human subjects at Brigham Young University. Before entering the study, all participants read and signed consent forms and completed a questionnaire pertaining to background information, medical history, and prescription medications. Subjects received $100 for participating in the study.

The specific days of health data collection for each panel along with PM$_{2.5}$ concentrations are presented in Figure 1. Multiple observations on the subjects were collected over the study periods. The days that health end point data were collected were not random because of the effort to collect health data for each subject at least once during periods of relatively high pollution and at least once during periods of relatively low pollution.

Pollution and weather data. Daily data for temperature and relative humidity were obtained for the Salt Lake City, Utah, International Airport monitoring station from the National Climatic Data Center (www.ncdc.noaa.gov). In addition, the National Weather Service computes an air stagnation index for the Wasatch Front. This index, also called the clearing index, is a profile of the atmosphere that incorporates temperature, moisture, and winds into an index that measures the vertical and horizontal motion of particles in the air (Jackman and Chapman 1977). The clearing index ranges from 0 to 1,050, where the values indicate the relative air stagnation. As climatic conditions lead to more stagnant air, the clearing index falls, and as conditions lead to less stagnant air, the clearing index rises, usually quite rapidly.

Daily 24-hr monitoring of airborne PM$_{2.5}$ was conducted by the State of Utah Division of Air Quality according to the U.S. Environmental Protection Agency’s (U.S. EPA) federal reference method (FRM; U.S. EPA 1997). Nonvolatile, PM$_{2.5}$ mass concentrations were determined using tapered element oscillating microbalance (TEOM) monitors (Patashnick and Rupprecht 1991). Total PM$_{2.5}$ mass, including semivolatile mass (SVM), was measured using two sampling methods. The first was a real-time total ambient mass sampler (RAMS), based on diffusion denuder, Nafion (Perma Pure LLC, Toms River, NJ) dryer, and TEOM monitor technology. This sampler has been described in detail elsewhere (Eatough et al. 1999, 2001). The second method used the Particle Concentrator-Brigham Young University Organic Sampling System (PC-BOSS) to measure fine particulate mass, crustal and trace elements, sulfate, carbonaceous material (elemental and organic), nitrate, semivolatile organic compounds, and semivolatile nitrate. The configuration and operation of the PC-BOSS as used in this study have been previously described in detail (Lewtas et al. 2001). These monitors were collocated with the State of Utah Division of Air Quality Hawthorne, Bountiful, and Lindon monitoring stations.

Because of the inability to predict pollution episodes more than a few days in advance and because of the complexity and experimental nature of some of the pollution monitoring, various scheduling and equipment failures meant that some pollution data were missing during blocks of time when health data were being collected. The most consistently reliable data were the PM$_{2.5}$ FRM data. However, even these data had some days missing. These missing data were estimated and filled in by extrapolation of the available data from the neighboring days.

**Figure 1.** PM$_{2.5}$ concentrations and number of observations of health end points for (A) Hawthorne winter, (B) Hawthorne summer, (C) Bountiful, and (D) Lindon. Black dots represent PM$_{2.5}$ data collected by the State of Utah Division of Air Quality according to the U.S. EPA’s FRM. Green dots represent missing data that were estimated using neighboring days and the clearing index. Bars represent the number of observations of health end points collected on given days. Black, red, green, yellow, and blue bars represent the first, second, third, fourth, and fifth time period of health data collection, respectively.
if there were no large changes in the clearing index, or by projection of data consistent with observed changes in the clearing index. As reported elsewhere (Eatough et al. 2003), the RAMS and PC-BOSS monitors provided nearly equivalent estimates of the PM$_{2.5}$ mass, including SVM for this analysis, the averages of the nonmissing values from 24-hr concentrations calculated from RAMS and PC-BOSS were used as estimates of total fine particulate mass. Figure 1 illustrates the PM$_{2.5}$ concentrations using FRM-filled and the time of health data collection for each panel. Figure 2 presents PM$_{2.5}$ measurements from FRM-filled, TEOM, and RAMS/PC-BOSS monitors plotted with the clearing index. Ambulatory ECG monitoring. Repeated 24-hr ambulatory ECG monitoring was conducted on the subjects during periods of both low and high air pollution. Participants were hooked up to the monitors by a trained technician in their homes. The hook-up of a modified V5 and VF bipolar lead placement were used, and skin preparation, electrode placement, and related protocols were similar to those described elsewhere (Marquette Medical Systems 1996). ECGs were recorded digitally (sampling rate, 256 Hz/channel) on removable flash cards using a lightweight, two-channel, ambulatory ECG monitor (Trillium3000; Forest Medical, East Syracuse, NY). The ECG digital recordings were processed, and mean heart rate and various measures of HRV were calculated for each 24-hr session using PC-based software (Trillium3000 PC Companion Software for MS Windows; Forest Medical). Three measures of HRV were calculated and used in this analysis: (a) SDNN, the standard deviation of all normal R-R (or NN) intervals during the 24-hr period; (b) SDANN, the standard deviation of the average NN intervals in all 5-min segments of the 24-hr period; and (c) r-MSSD, the square root of the mean of the squared differences between adjacent NN intervals. SDNN is an estimate of overall HRV, SDANN reflects variability due to cycles longer than 5 min and is an estimate of long-term components of HRV, and r-MSSD reflects high-frequency variations and is an estimate of short-term components of HRV. A detailed discussion of the physiologic interpretation of these measures is presented elsewhere (Task Force 1996).

Blood collection and analysis. Immediately after each 24-hr ECG monitoring period, blood was drawn using standardized procedures for venipuncture, collection, storage, and shipment. Blood cell counts and differential white cell counts were determined using an automated cell counter with flow differential (Advia 120 hematology system; Bayer Corporation, Leverkusen, Germany); C-reactive protein (CRP) was measured using nephelometry (IMMAGE Immunochemistry Systems, CRP Kit 447280; Beckman Coulter Instruments, Brea, CA); whole blood viscosity was measured using a cone-plate viscometer (model DZ-2+; Brookfield Engineering Laboratories Inc., Middleton, MA). All of the blood tests were conducted at ARUP Laboratories in Salt Lake City.

Analytic methods. As illustrated in Figure 1, we attempted to collect health data during times of both high and low PM$_{2.5}$ concentrations. Associations for each of the heart rate, HRV, and blood measures were evaluated statistically using various regression models. To account for subject-specific differences two approaches were used: (a) Deviations from each individual subject’s mean were calculated and used as the dependent variables in the regression models, and (b) subject-specific fixed effects (Greene 2000) were included in the regression models. Three approaches were used to control for weather variables: (a) Both linear and quadratic terms for daily average temperature and relative humidity were included in the models, (b) additive cubic smoothing splines with 3 degrees of freedom (df) for average temperature and relative humidity were included in the models, and (c) cubic smoothing splines with a functional two-way interaction between average temperature and relative humidity using 6 df were included in the models. Mean heart rate was included in some of the models for SDNN, SDANN, and r-MSSD. Although most of the models included PM$_{2.5}$ as a linear term, models that included cubic smoothing splines with 3 df for PM$_{2.5}$ were also estimated. The statistical analysis was conducted using SAS statistical software (SAS Institute 2001) estimating the fully parametric regression models using PROC REG and estimating the regression models that included cubic smoothing splines using the “spline” and “spline2” functions in the model statement of PROC GAM.

Figure 2. PM$_{2.5}$ measurements for (A) Hawthorne winter, (B) Hawthorne summer, (C) Bountiful, and (D) Lindon; from FRM-filled (thick black lines), RAMS/PC-BOSS (red), and TEOM (blue) monitors plotted with the clearing index (thin black lines).
We conducted residual analysis of the estimated regression models by labeling residuals and partial residuals by subject number and plotting them over pollution levels. We then evaluated the sensitivity of the results to influential subjects by estimating the regression models with the influential subjects excluded. Also, we estimated models for different lagged days of exposure and for PM2.5 concentrations measured by the alternative monitoring approaches.

Results
Summary statistics for the pollution, weather, and health variables are presented in Table 1. As Table 1 and Figure 1 show, during the study period PM2.5 pollution levels rarely exceeded the U.S. National Ambient Air Quality 24-hr standard of 65 µg/m³ (U.S. EPA 1996, 1997). The mean levels of the HRV and blood measures were not remarkable.

Table 2 presents the regression coefficients for five alternative regression models. Elevated concentrations of PM2.5 were consistently associated with declines in all three measures of HRV, SDNN, SDANN, and r-MSSD. As expected, there were substantial cross-subject differences in these HRV measures. There were also statistically significant associations between HRV and temperature and relative humidity.

Table 1. Summary statistics of key variables used in analysis.

| Variable          | Unit            | Reference range | No. | Mean ± SD | Min–Max |
|-------------------|-----------------|-----------------|-----|-----------|---------|
| PM2.5 (FRM-Filled) | µg/m³           | —               | 250 | 23.7 ± 20.2 | 1.7–74.0 |
| PM2.5 (not filled) | µg/m³           | —               | 182 | 25.8 ± 21.2 | 1.7–74.0 |
| PM2.5 (TEOM)      | µg/m³           | —               | 223 | 18.9 ± 13.4 | 2.2–61.5 |
| PM2.5 (RAMS/PC-BOSS) | µg/m³        | —               | 236 | 26.5 ± 18.8 | 5.6–72.4 |
| Average temperature | °F              | —               | 250 | 43.3 ± 20.8 | 19.0–87.0 |
| Average relative humidity | %       | —               | 250 | 67.5 ± 18.9 | 24.5–92.0 |
| Mean heart rate   | bpm             | —               | 247 | 72.1 ± 9.8  | 50–104  |
| SDNN              | msec            | —               | 246 | 131.4 ± 43.1| 45–317  |
| SDANN             | msec            | —               | 246 | 109.0 ± 38.1| 37–376  |
| r-MSSD            | msec            | —               | 246 | 68.7 ± 59.5 | 14–323  |
| CRP               | mg/dL           | 0.0–0.8         | 244 | 0.50 ± 0.60 | 0.10–7.8 |
| Whole blood viscosity | cP           | 3.6–6.0         | 248 | 5.15 ± 0.76 | 0.80–8.3 |
| White blood cells  | k/µL            | 3.2–10.6        | 250 | 6.99 ± 1.59 | 3.60–14.11 |
| Granulocytes      | k/µL            | 1.3–7.0         | 237 | 4.26 ± 1.20 | 2.10–11.10 |
| Lymphocytes       | k/µL            | 0.8–3.1         | 237 | 2.14 ± 0.72 | 0.50–4.80 |
| Monocytes         | k/µL            | 0.1–0.5         | 237 | 0.41 ± 0.16 | 0.10–1.30 |
| Basophils         | k/µL            | 0.0–0.1         | 237 | 0.05 ± 0.05 | 0.00–0.20 |
| Eosinophils       | k/µL            | 0.0–0.4         | 237 | 0.19 ± 0.11 | 0.00–0.70 |
| Red blood cells   | M/µL            | 4.69–6.07       | 250 | 5.74 ± 0.43 | 3.32–6.16 |
| Platelets         | k/µL            | 177–406         | 250 | 249.3 ± 58.7| 101.0–467.0 |

Abbreviations; bpm, beats per minute; cP, centipoise; k/µL, thousands per microliter; Max, maximum; Min, minimum; M/µL, millions per microliter.

Table 2. PM2.5 regression coefficients (×100 [SEs]) for various regression models using all subjects and FRM-filled PM2.5 data.

| Model parameters  | I              | II             | III            | IV             | V              |
|-------------------|----------------|----------------|----------------|----------------|----------------|
| Control for cross-subject differences | Dependent variables are deviations from individual means | Subject-specific fixed effects | Subject-specific fixed effects | Subject-specific fixed effects | Subject-specific fixed effects |
| Control for other factors | Quadratic functions for PM2.5 | Quadratic functions for temp, RH | Additive spline smooths for temp, RH | Additive spline smooths for temp, RH plus control for HR | Interactive spline smooths for temp, RH; partial control for HR |
| Mean heart rate   | –3.83 (2.5)    | –5.23 (3.47)   | –5.14 (2.42)** | –5.14 (2.42)** | –5.14 (2.42)** |
| SDNN              | –31.45 (12.27)** | –37.76 (17.01)** | –34.36 (12.13)* | –41.71 (11.67)** | –34.94 (10.32)** |
| SDANN             | –16.80 (12.55) | –19.92 (17.46) | –17.42 (12.43) | –22.93 (12.20)* | –18.98 (8.67)** |
| r-MSSD            | –39.31 (15.64)** | –50.59 (21.80)** | –45.82 (15.42)# | –51.15 (15.27)# | –42.25 (10.90)# |
| CRP               | 0.78 (0.25)    | 0.95 (0.35)    | 0.96 (0.25)    | 0.96 (0.25)    | 0.96 (0.25)    |
| Whole blood viscosity | 0.15 (0.55)    | 0.25 (0.75)    | 0.21 (0.53)    | 0.21 (0.53)    | 0.21 (0.53)    |
| Granulocytes      | 0.21 (0.53)    | 0.38 (0.72)    | 0.33 (0.51)    | 0.33 (0.51)    | 0.33 (0.51)    |
| Lymphocytes       | –0.06 (0.20)   | –0.09 (0.28)   | –0.06 (0.20)   | –0.06 (0.20)   | –0.06 (0.20)   |
| Monocytes         | 0.13 (0.06)**  | 0.15 (0.08)**  | 0.14 (0.08)**  | 0.14 (0.08)**  | 0.14 (0.08)**  |
| Basophils         | –0.02 (0.02)   | –0.02 (0.03)   | –0.02 (0.02)   | –0.02 (0.02)   | –0.02 (0.02)   |
| Eosinophils       | –0.03 (0.03)   | –0.03 (0.04)   | –0.02 (0.03)   | –0.02 (0.03)   | –0.02 (0.03)   |
| Red blood cells   | 0.04 (0.09)    | 0.03 (0.13)    | 0.00 (0.09)    | 0.00 (0.09)    | 0.00 (0.09)    |
| Platelets         | 0.58 (13.33)   | –1.18 (18.49)  | 2.29 (13.14)   | 2.29 (13.14)   | 2.29 (13.14)   |

Abbreviations; RH, relative humidity; temp, temperature.

*Includes linear and quadratic terms for average temperature and relative humidity. **Includes cubic smoothing splines with 3 df for average temperature and relative humidity. ***Includes cubic smoothing splines with a functional two-way interaction between average temperature and relative humidity using 6 df. Models for SDNN, SDANN, and r-MSSD also includes mean heart rate. *p ≤ 0.10; **p ≤ 0.05; ***p ≤ 0.01.
residual plots not shown). For the measures of HRV (only SDNN shown in Figure 3), two subjects (89 and 66) appeared to be most influential, with two other subjects (36 and 77) successively less so. For the other end points, no influential subjects were clearly observed.

Table 3 presents the PM$_{2.5}$ regression coefficients using model V excluding various numbers of influential subjects. For the measures of HRV, excluding influential subjects produced somewhat smaller estimated PM$_{2.5}$ effects, but the effects remained negative and generally statistically significant. For CRP, excluding the single highly influential subject produced a substantially smaller estimated PM$_{2.5}$ effect that was marginally significant (p = 0.06). For mean heart rate and monocytes, excluding the influential subjects resulted in reduced and statistically insignificant associations with PM$_{2.5}$.

As illustrated for SDNN and CRP in Figure 3, associations with PM$_{2.5}$ were nearly linear. Goodness-of-fit tests, based on models that included cubic smoothing splines with 3 df for PM$_{2.5}$, indicated that PM$_{2.5}$ associations with r-MSSD but not SDNN, SDANN, or CRP were significantly different from linear (p < 0.05). However, after influential observations were removed, no associations between PM$_{2.5}$ and SDNN, SDANN, r-MSSD, or CRP were significantly different from linear (p > 0.25).

Table 4 presents regression coefficients with model V using different lagged days of exposure. The strongest associations between PM$_{2.5}$ and HRV were with concurrent-day exposures. The strongest associations between PM$_{2.5}$ and CRP were with model V using different lagged days of exposure. Table 4 shows associations with PM$_{2.5}$ were nearly linear with effect estimates generally decreasing for longer exposure lag times. For CRP, the concurrent-day exposures were also most strongly associated with CRP, but a relatively large effect was also observed for the 3 days’ previous exposure.

As shown in Figures 1 and 2, for all measures of PM$_{2.5}$ during prolonged periods with a very low and stable clearing index (indicating very stagnant air conditions), PM$_{2.5}$ concentrations tended to rise and then drop rapidly with the eventual and inevitable rapid rise in the clearing index. Using model V, measures of HRV and CRP were also regressed on other estimates of concentrations of PM$_{2.5}$, including FRM without filling in missing values and the TEOM and RAMS/PC-BOSS measures (results not shown). For the FRM (not filled) and for TEOM, PM$_{2.5}$ effect estimates were nearly identical to those reported in Table 2. For the PM$_{2.5}$ concentrations measured using RAMS/PC-BOSS that included SVM, the results were similar for CRP, but statistically significant negative associations were not observed for measures of HRV. However, there were 10 days with abnormally high levels of SVM (> 15 µg/m$^3$), caused by abnormally elevated concentrations of ammonium nitrate. When these 10 days were deleted from the analysis, statistically significant negative associations, similar to those presented in Table 2, were observed for PM$_{2.5}$ from the RAMS/PC-BOSS monitors.

**Discussion**

In this study we hypothesized that altered cardiac autonomic function, as indicated by changes in measures of HRV, and pulmonary inflammation, as measured by various blood markers of inflammation, would be associated with ambient concentrations of PM$_{2.5}$. Reasonably consistent and statistically robust negative associations between PM$_{2.5}$ and measures of HRV were observed. PM$_{2.5}$ was significantly associated with CRP but not with changes in white blood cell counts, red blood cells, platelets, or whole-blood viscosity. The PM$_{2.5}$ associations with CRP are interesting and suggestive, but given that they depend largely on a highly influential subject, they are not compelling.

Previous studies have reported HRV associations with ambient PM air pollution exposure (Gold et al. 2000; Liao et al. 1999; Pope et al. 1999), occupational PM exposure (Magari et al. 2001), and PM exposure from secondhand cigarette smoking in an airport smoking lounge (Pope et al. 2001). A previous study that used 24-hr ambulatory ECG monitoring was also conducted along the Wasatch Front in Utah (Pope et al. 1999), but it had only seven participants with a total of 39 observations and had air pollution monitoring only for PM ≤ 10 µm in aerodynamic diameter (PM$_{10}$). This study estimated that a 100-µg/m$^3$ increase in PM$_{10}$ was associated with an 18-msec decrease in 24-hr SDNN. Assuming a PM$_{2.5}$:PM$_{10}$ ratio of 0.60, this would suggest a 30-msec decline in 24-hr SDNN associated with a 100-µg/m$^3$ increase in PM$_{2.5}$—an estimate remarkably close to that observed in this study. However, unlike in the present analysis, PM pollution was associated with increases in 24-hr r-MSSD. Another study conducted in Boston, Massachusetts, estimated changes in white blood cell counts, red blood cells, platelets, or whole-blood viscosity. The PM$_{2.5}$ associations with CRP are interesting and suggestive, but given that they depend largely on a highly influential subject, they are not compelling.

**Table 3.** PM$_{2.5}$ (FRM-filled) regression coefficients [×100 (SEs)] using model V*, excluding various numbers of influential subjects.

| Excluding all subjects | Excluding two influential subjects | Excluding three influential subjects | Excluding four influential subjects |
|------------------------|-----------------------------------|-------------------------------------|-----------------------------------|
| Mean heart rate | –1.86 (1.55) | –22.70 (7.13)$^*$ | –31.01 (6.75)$^*$ | –26.39 (6.84)$^*$ |
| SDNN | –0.00 (0.10) | –14.37 (6.96)$^*$ | –23.09 (6.31)$^*$ | –19.94 (6.41)$^*$ |
| SDANN | –25.63 (8.95)$^*$ | –34.96 (8.45)$^*$ | –21.77 (7.96)$^*$ |
| r-MSSD | –23.70 (7.13)$^*$ | –31.01 (6.75)$^*$ | –26.39 (6.84)$^*$ |
| CRP | –0.02 (0.03) | –14.37 (6.96)$^*$ | –23.09 (6.31)$^*$ | –19.94 (6.41)$^*$ |
| Monocytes | 0.02 (0.03) | –23.09 (6.31)$^*$ | –19.94 (6.41)$^*$ |

*This model included subject-specific fixed effects and cubic smoothing splines with a functional two-way interaction between average temperature and relative humidity using 6 df. Models for SDNN, SDANN, and r-MSSD also included mean heart rate. These models excluded highly influential subjects as determined by residual analysis. Only one subject was excluded for mean heart rate (subject 77), CRP (15), and monocytes (9). For the HRV measures, SDNN, SDANN, and r-MSSD, initially two subjects were excluded (66 and 89), then three (36, 66, and 89), and then four (36, 66, 89, 77). $^*$p ≤ 0.10; $^{**}$p ≤ 0.05; $^{***}$p ≤ 0.01.

**Table 4.** PM$_{2.5}$ (FRM-filled) regression coefficients [×100 (SEs)] using model V* using different lagged days of exposure.

| Day | Concurrent day | Previous day | Two days previous | Three days previous |
|-----|---------------|-------------|-------------------|---------------------|
| SDNN | –34.94 (8.32)$^*$ | –19.37 (9.21)$^{**}$ | –23.70 (7.13)$^*$ | –26.39 (6.84)$^*$ |
| SDANN | –18.98 (8.67)$^{**}$ | –10.22 (9.51) | –6.80 (9.94) | –1.52 (10.43) |
| r-MSSD | –42.25 (10.90)$^*$ | –27.66 (12.04)$^{**}$ | –27.66 (12.04)$^{**}$ | –27.66 (12.04)$^{**}$ |
| CRP | 0.81 (0.18)$^*$ | 0.33 (0.20)$^*$ | 0.38 (0.20)$^*$ | 0.64 (0.21)$^*$ |

*This model included subject-specific fixed effects and cubic smoothing splines with a functional two-way interaction between average temperature and relative humidity using 6 df. Models for SDNN, SDANN, and r-MSSD also included mean heart rate. $^*$p ≤ 0.10; $^{**}$p ≤ 0.05; $^{***}$p ≤ 0.01.
the effect of average PM$_{2.5}$ levels over the previous 4 hr on SDNN and r-MSSD during a 25-min period of alternating rest and exercise in a panel of 21 participants 53–87 years of age with a total of 163 monitoring sessions (Gold et al. 2000). A 100-µg/m$^3$ increase in exposure to PM$_{2.5}$ was associated with an approximately 24-msec decline in both SDNN and r-MSSD.

The physiologic importance of these observed changes in HRV may increase the likelihood of sudden cardiac death (Task Force 1996). In addition, decreases in HRV are strong predictors of mortality (Kennedy 1997; La Rovere et al. 2003), and autonomic nervous system–activated changes in HRV may reflect increased risk for adverse cardiovascular events.

The suggested associations between PM$_{2.5}$ and CRP are intriguing. The MONICA-Augsburg study also reported evidence of increased CRP associated with PM air pollution (Peters et al. 2001). A 3-fold increase in the odds of having CRP > 5.7 mg/L was associated with a marked pollution episode. Using multivariate regression analysis, the study also found an increase in CRP of 0.88 mg/L associated with an increase of 2 µg/m$^3$ in the previous 5-day average concentration of total suspended particles, which converts to a CRP increase of 0.34 mg/dL for a 100-µg/m$^3$ increase in the 5-day average total suspended particles. This estimated change is roughly comparable with changes observed in the present analysis. Seaton et al. (1999) also reported a positive association between PM$_{10}$ and CRP. A 147% (95% confidence interval, 20–477) increase in CRP was associated with a 100-µg/m$^3$ increase in PM$_{10}$.

Recently, much attention has been placed on CRP as an indicator of acute phase response and as a risk factor for cardiovascular events. Remarkably small changes in CRP have been associated with changes in cardiac risk. In a study by Ridker et al. (2002) involving approximately 28,000 American women, the relative risk of a first cardiovascular event increased with higher quintiles of CRP. The CRP range for the fourth quintile reported by Ridker et al. (2002) was 2.09–4.19 mg/L. The median CRP in our present study (0.4 mg/dL or 4 mg/L), therefore, would fall within the upper range of this fourth quintile. Also, the estimated increase in CRP associated with a 100-µg/m$^3$ increase in PM$_{2.5}$ reported in this study, even excluding the highly influential observation (0.19 mg/dL or 1.9 mg/L), was approximately equal to or greater than the range of the CRP quintiles reported by Ridker et al. (2002). However, it is uncertain how these acute changes in CRP effect or reflect potential changes in cardiovascular risk.

The analysis using the RAMS/PC-BOSS data observed that the PM–HRV associations were much smaller on days with high SVM, especially if the SVM was rich in nitrate. These results are not well understood, but they imply that nitrate may have a smaller impact than to do other fine particles or that nitrate exposure is not well accounted for by central-site monitoring (Patterson and Eatough 2000). The health associations for PM$_{2.5}$ measured by TEOM (which includes no SVM), PM$_{2.5}$ measured by FRM (which includes some SVM), and PM$_{2.5}$ measured by RAMS/PC-BOSS (which is designed to measure total PM including SVM) after deleting days with abnormally high levels of ammonium nitrate were all similar. These results suggest that organic semivolatile may have effects similar to those of the nonvolatile PM and point toward the need for further particle characterization in health studies of PM pollution. Additional study with other pollution measures including gaseous pollutants may be important.

Although in this study we observed statistical associations between PM$_{2.5}$ and HRV and CRP, most of the relevant variability in the temporal deviations of these physiologic end points was not explained by PM$_{2.5}$. The fact that small but statistically significant associations were observed suggests that PM$_{2.5}$ may be one of multiple factors that influence HRV and CRP. These results suggest that altered cardiac autonomic function and pulmonary/systemic inflammation may play a role in pathogenesis relating to the harmful effects of PM air pollution on human health.

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