PM2.5-induced cardiovascular dysregulation in rats is associated with elemental carbon and temperature-resolved carbon subfractions

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Abstract

Background: We tested the hypothesis that cardiovascular responses to PM2.5 exposure will be enhanced in hypertensive rats and linked to specific carbonaceous pollutants in an urban industrial setting.

Methods: Spontaneously hypertensive rats were exposed by inhalation to concentrated PM2.5 in an industrial area of Dearborn, Michigan, for four consecutive summer days. Blood pressure (BP), heart rate (HR) and HR variability (HRV) metrics (SDNN, RMSSD) were assessed by radiotelemetry and compared to 1 h- and 8 h-averaged fluctuations in PM2.5 composition, with a focus on elemental and organic carbon (EC and OC, respectively), and temperature-resolved subfractions (EC1-EC5, PC (pyrolyzed carbon), and OC1-OC4), as well as other major and minor PM components.

Results: Mean HR and BP were increased, while HRV was decreased over 4 days of exposure. Using 1 h averages, EC (1 μg/m³ increase) was associated with increased HR of 11-32 bpm (4-11% increase), 1.2-1.5 ms (22-27%) decreases in SDNN, 3-14 mmHg (1.5-8%) increases in systolic BP, and 5-12 mmHg (4-9%) increases in diastolic BP. By comparison, associations with OC were negligible. Using 8 h averages, EC subfractions were linked with increased heart rate (EC1: 13 bpm; EC2, EC3, PC: <5 bpm) and SDNN (EC1 > EC2 > EC3, EC4, PC), but with decreased RMSSD (EC2, EC5 > EC3, EC4). Minimal effects were associated with OC and OC1. Associations between carbon subfractions and BP were negligible.

Conclusions: These findings are the first to describe associations between acute cardiovascular responses and thermally resolved carbon subfractions. We report that cardiovascular responses to PM2.5 carbonaceous materials appear to be driven by EC and its EC1 fraction.

Keywords: Air pollution, Blood pressure, Elemental carbon, Heart rate, Inhalation, Organic carbon, PM2.5, Rat
In addition to associations with PM$_{2.5}$ mass, two recent meta-analyses using Medicare records and particle composition data from the Environmental Protection Agency Chemical Speciation Network found significant associations between elemental carbon (EC), a combustion product most often linked to diesel engine exhaust, and hospital admissions for cardiovascular causes [11,12]. Ambient EC concentrations have been associated with increased systolic pressure [13-15] and HRV [13,16]. Another major carbonaceous fraction of PM$_{2.5}$ is organic carbon (OC), which is derived from mobile sources, biomass burning and industrial processes and has also been linked to decreased HRV and elevated BP [6,16]. Extensive analyses of PM$_{2.5}$ EC and OC using thermal/optical analytical approaches yields up to five OC subfractions (OC1-OC4 and pyrolyzed carbon [OP]) and four EC subfractions (EC1-EC4). This method applies step-wise increases in temperature in a controlled atmosphere to oxidize first organic and then elemental carbon samples to yield eight fractions with decreasing volatility and increasing structural complexity. Thus OC1 and EC1 are more volatile and simpler in structure than OC4 and EC4, respectively. These carbon subfractions have been used to refine source apportionment analysis in the U.S. [17] and more recently in China [18,19]; however, the relative health effects of these temperature-resolved subfractions of EC and OC have not been evaluated.

We have previously reported altered HRV in hypertensive rats exposed to concentrated PM$_{2.5}$ in Detroit, MI and Steubenville, OH that was linked to specific PM components and related industrial and mobile sources [20,21]. In these studies we found EC to consistently have the strongest association of any PM$_{2.5}$ component with changes in heart rate and HRV. Our goal in the present study was to extend these observations to include blood pressure responses in hypertensive rats, as well as to provide a detailed characterization of both EC and OC and their subfractions in an industrial urban center in Dearborn, MI. Our results describe clear differences between EC and OC in PM$_{2.5}$-induced responses, as well as provide initial insights into the relative potencies of carbon subfractions in relation to acute cardiovascular responses.

**Results**

**Exposure characterization**

The average ($\pm$SD) chamber PM$_{2.5}$ mass concentrations during the four 4-day exposure studies were as follows: Study 1, 415 ± 99; Study 2, 642 ± 294; Study 3, 767 ± 256; Study 4, 364 ± 58 μg/m$^3$. Figure 1 depicts the distribution of major chemical components of PM$_{2.5}$ collected during each 4-day inhalation exposure period. Table 1 shows the combined numeric average for all exposures. As previously observed in this Dearborn study area [22,23], sulfate and OC dominated during the summer months. Figure 2 shows the average carbon fraction distribution of concentrated PM$_{2.5}$ during the exposures. EC3 & EC4 were the most prevalent EC subfractions, while OC1 & OC4 subfractions were the most prevalent species within OC.

**Effect of PM$_{2.5}$ exposure on cardiovascular responses**

Comparisons of responses in rats exposed to AIR vs. PM$_{2.5}$ using 1 h averaged data resulted in several-exposure-related differences (Table 2). Changes in blood pressure responses were the most sensitive indicator of PM$_{2.5}$ exposure, with significant associations with MAP, systolic and diastolic pressures during 3 of the 4 exposure studies. Other significant associations were found for heart rate during studies 1 & 3, and SDNN during Study 3.

**Associations with major components and trace elements**

Assessment of major non-carbonaceous PM components (nitrates, sulfates and ammonia) and 29 elements revealed a number of minor, but statistically significant associations with HR, HRV and BP (Table 3, Additional file 1: Tables S1 and S2). For example, significant responses of less than 0.1 bpm for HR and less than 0.01 ms for lnSDNN were associated with the change in IQR for a few specific components or elements. The only PM$_{2.5}$ constituent with a larger effect estimate was uranium, which was associated with a 15 mm Hg increase in diastolic pressure (Table 3), although the associations between this element and both systolic BP and MAP were not significant (Additional file 1: Table S2). The size of the uranium effect estimate compared to other components may be due to its low ambient concentration (0.19 ng/m$^3$), and relative IQR (0.2 ng/m$^3$).

**Associations with carbon fractions**

During all four exposure studies, PM$_{2.5}$ EC concentration was consistently associated with increased HR with estimated changes of 11-32 bpm (4-11% increase) for a 1 μg/m$^3$ increase in EC (Table 4). EC was also associated with 22-27% decreases in HRV during Studies 1 & 4. By comparison, OC and PM$_{2.5}$ mass had little to no associations with cardiac automaticity.

Changes in blood pressure also appeared to be more influenced by variations in EC than by OC or PM mass (Table 5). Increases in systolic BP ranged from 3-14 mmHg (1.5-8% increases) for a 1 μg/m$^3$ increase in EC for Studies 1-4. MAP was elevated by 4-9% in Studies 1 & 2 while diastolic BP increased significantly with EC by 4-10% in three of the four exposure studies.

**Associations with carbon subfractions**

Overall the daily concentrations of EC subfractions had greater associations with heart rate and HRV changes than OC subfractions (Figure 3A-C). Subfractions EC1, EC2, and EC3 were all significantly associated with increased heart rate and SDNN, with EC1 having the largest effect estimate (HR increase of 13 bpm; SDNN increase of...
approximately 7.5%). Interestingly, no effects were found for EC itself using the 8 h averages. EC2, EC3, EC4, and EC5 were all significantly negatively associated with reductions in RMSSD. EC1 showed extremely wide confidence intervals. Small effect estimates were seen for OC and OC1, the only organic carbon fractions with significant associations. Carbon subfractions had weak associations with vascular responses (Figure 3D-F). Except for total EC which had small effect estimates (>1 mmHg) for both MAP and DBP, no EC or OC subfractions were significantly associated with any change in blood pressure.

**Discussion**

Results of this study clearly demonstrate that the EC and EC subfractions drive the acute changes in HR, HRV and BP in hypertensive rats exposed to an urban-industrial aerosol. These findings are consistent with our previous report of EC’s relation with HR in the same rat model [21], but we now extend these observations to identify EC-associated effects on BP as well, suggesting a more profound overall impact on cardiovascular health. Furthermore, we detected associations between EC subfractions and cardiac function, especially between EC1 and EC2 with HR and HRV. By comparison to EC, OC was associated with only modest and sometimes opposing cardiovascular effects in PM2.5-exposed rats. To our knowledge, our current findings are the first to describe associations of cardiovascular health effects with inhaled PM2.5 carbon subfractions using thermal/optical analytical methods.

Although not all source apportionment studies include EC and OC in their factor resolution, those that do typically find that these PM components load onto mobile source emissions factors [24,25]. Furthermore, the separation of carbonaceous fractions derived from progressive oxidation temperatures suggest that the subfraction EC1 is generally linked to diesel engine sources, whereas OC fractions (OC1-4) are normally associated with gasoline emissions [17,26]. It is notable, therefore, that the adverse cardiovascular responses we describe for EC and its

**Figure 1** Distribution of major components in concentrated PM$_{2.5}$ during exposures. Concentrations of organic matter (OM: OC × 1.8), elemental carbon (EC), sulfate, nitrate; ammonia, crustal elements and other components were determined from 8 h samples from the four separate field studies as described in Materials and Methods. Crustal = 2.14Al + 2.43Fe + 1.54Si, where Si was estimated as potassium (K)/0.15.

**Table 1 Average mass of PM$_{2.5}$ constituents in chambers during inhalation exposures**

| Constituent     | Mass (SEM) (mg/m3) |
|-----------------|--------------------|
| PM$_{2.5}$      | 547 ± 62.3         |
| OM              | 208 ± 15.3         |
| EC              | 10 ± 1.3           |
| Sulfate         | 154 ± 28.8         |
| Nitrate         | 24 ± 5.5           |
| Ammonium        | 52 ± 11.9          |
| Urban dust      | 56 ± 8.1           |
| Other           | 58 ± 14.7          |

Data represent the daily mean of 16 days of exposures (four studies of 4 days each). OM-organic matter; EC – elemental carbon.
subfractions are consistent with HR, HRV and BP responses reported during controlled exposures to whole diesel engine exhaust in the same SH strain of rats we used in the current study [27,28]. While major components of laboratory-generated diesel exhaust are gaseous inorganic compounds (nitrogen oxides, sulfur dioxide, and carbon monoxide), the minor components of particulate EC and of volatile and semivolatile organic compounds (OC) have been linked to stimulation of both sympathetic and parasympathetic cardiovascular effects in SH rats where exposures compared whole versus filtered exhaust [29,30]. A major difference in our field studies compared to laboratory-generated diesel engine exhaust is the atmospheric transformation of EC core particulates that might result in surface adherence of volatile organic hydrocarbons such as carbonyls, or in oxidative modifications that alter the particle’s toxicity [31,32]. OC was a major component of PM2.5 mass at our urban site, and though not strongly associated with health effects in our study, it may contribute to cardiovascular effects as a surface component of diesel soot particles.

Our study location in Dearborn, MI is located near automotive production industries with heavy vehicle and diesel traffic in the surrounding community. Of note are several active trucking facilities within a mile of the site, with several hundred trucks loading and unloading cargo daily, as well as a railyard within 250 m of the site. We recently compared cardiometabolic responses in twenty-five volunteers before, during and after ambient exposures at this same industrial site, relative to their residences in rural upwind areas of Dexter, MI [33]. After five daily exposures, decreases in HRV and insulin resistance were associated with increased PM2.5. Further analyses of source: health effect relationships found that changes in HR, BP and trends for impaired endothelial function were associated with the diesel source factor that impacted this site [34]. Fewer health effects were associated with other PM source factors, with motor vehicle sources being linked to changes in BP, and iron/steel and secondary aerosol source factors being associated with changes in HR. We recently reported dramatic drops in BP and HR in fructose-fed rats with cardiometabolic syndrome exposed to PM2.5 at this same site in Dearborn; however, analyses to attribute specific sources with CV responses have not been completed.

### Table 2: Effect of PM$_{2.5}$ exposure on cardiovascular responses in SH rats

| Response | Study | dN | dfD | p-value |
|----------|-------|----|-----|---------|
| Heart rate | 1 | 35 | 421 | *0.0246 |
| | 2 | 35 | 455 | 0.365 |
| | 3 | 35 | 493 | *0.0008 |
| | 4 | 35 | 504 | 0.661 |
| lnSDNN | 1 | 35 | 421 | 0.591 |
| | 2 | 35 | 455 | 0.061 |
| | 3 | 35 | 493 | *0.0372 |
| | 4 | 35 | 504 | 0.356 |
| lnRMSSD | 1 | 35 | 421 | 0.076 |
| | 2 | 35 | 455 | 0.546 |
| | 3 | 35 | 493 | 0.289 |
| | 4 | 35 | 504 | 0.266 |
| MAP | 1 | 35 | 391 | *0.0124 |
| | 2 | 35 | 456 | *0.0107 |
| | 3 | 35 | 495 | *0.0106 |
| | 4 | 35 | 504 | 0.459 |
| Systolic | 1 | 35 | 391 | *0.0239 |
| | 2 | 35 | 456 | *0.0127 |
| | 3 | 35 | 495 | *0.0193 |
| | 4 | 35 | 504 | 0.476 |
| Diastolic | 1 | 35 | 391 | *0.0085 |
| | 2 | 35 | 456 | *0.0097 |
| | 3 | 35 | 495 | *0.0059 |
| | 4 | 35 | 504 | 0.415 |

Data are expressed as p-values for the interaction of Exposure (Air vs. PM$_{2.5}$) and time (1 h intervals). dN- degrees freedom for subjects, dfD degrees freedom for observations. *p < 0.05.

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**Figure 2** Distribution of EC and OC subfractions during exposures. Concentrations in 8 h samples of subfractions of elemental carbon (EC) and organic carbon (OC) were determined by thermal/optical approaches as described in Materials and Methods.
Table 3: Associations between major non-carbonaceous PM components and trace elements and diastolic blood pressure

| Component   | Effect (mmHg) | SEM  | p-value | Change per 1 ng/m³ | CI (lower, upper) | IQR (ng) |
|-------------|---------------|------|---------|--------------------|------------------|----------|
| Uranium     | 15.22         | 7.44 | 0.0434  |                    |                  |          |
| Rubirium    | 0.146         | 0.05 | 0.0081  |                    |                  |          |
| Crustal     | 0.076         | 0.03 | 0.0153  |                    |                  |          |
| Manganese   | 0.006         | 0.00 | 0.0465  |                    |                  |          |
| Aluminum    | 0.002         | 0.00 | 0.0407  |                    |                  |          |
| Potassium   | 0.0017        | 0.00 | 0.0157  |                    |                  |          |
| Magnesium   | 0.0014        | 0.00 | 0.0487  |                    |                  |          |
| Iron        | 0.0005        | 0.00 | 0.0264  |                    |                  |          |

Data are expressed as change in response (effect) per IQR of pollutant, and per 1 ng/m³.

[35]. Our findings with EC and subfractions in the current study add to our previous work with PM2.5-exposed SH rats where EC and traffic sources had robust associations with changes in HR and HRV in urban Detroit, MI and Steubenville, OH [20,21].

To date, carbon subfraction analysis has been employed in air pollution studies primarily to incorporate fractions into source apportionment analyses and, ideally, to improve source factor resolution [36,37]. For the most part, such studies have also been able to differentiate gasoline from diesel emissions, as discussed above. In addition, some work has investigated indoor and non-indoor sources of carbon fractions in residential homes [38]. We were able to identify one in vitro study including carbon subfractionation in which human lung epithelial cells were exposed to dust from soil and road surfaces in the western United States [39]. Release of inflammatory mediators was most highly correlated with the EC1 fraction, while lesser correlation coefficients were observed for OC fractions and pyrolyzed carbon. Compared to other EC fractions, compounds that comprise EC1 and EC2 would be more volatile, of smaller molecular weight, and a less complex structure. Less clear are the molecular targets, receptors or proteins with which different subfractions may preferentially interact to elicit biological responses. In addition to the greater responses induced in airway cells, smaller sized EC1 compounds would theoretically be better able to translocate and influence extrapulmonary responses. However, epidemiological or clinical evidence for EC subfraction-associated health effects is lacking, and we speculate that EC and EC1 may be markers for other pollutants or pollutant mixtures that underlie the health effects we describe in exposed rats.

In summary, this is the first report of cardiovascular health effects linked to inhalation exposure to ambient PM$_{2.5}$ carbon subfractions. Increased BP and HR and decreases in HRV showed robust associations with EC, and our initial findings using thermal optical approaches yielded strong relationships of EC1 and EC2 with changes in HR and HRV. Interestingly, we found relatively fewer and weaker responses with OC fractions or trace elements. Black carbon has been proposed as an important indicator of PM-induced health effects [43], and our results with EC add to this evidence, specifically for adverse cardiovascular responses. Future research efforts that include the analyses of carbon subfractions are needed to confirm our observations, and will help to further characterize the contribution of EC to the health risk of PM$_{2.5}$ exposures.
Methods

Animals
Sixty-four male spontaneously hypertensive (SH) rats 12-13 weeks of age (Charles River Laboratories, Portage, MI) were initially housed in animal facilities at Michigan State University (MSU) until moved to the mobile lab, where they were placed individually in polycarbonate cages on corn cob bedding with ad libitum access to food and water. Study protocols were approved by the Institutional Animal Care and Use Committee of MSU, an AAALAC accredited institution.

Exposure to PM$_{2.5}$
Inhalation exposures were conducted in AirCARE 1, a mobile air research laboratory parked at Salinas Elementary School in Dearborn, MI during the summers of 2009 and

| Table 4 Associations between PM$_{2.5}$ mass, EC and OC and cardiac responses |
|-----------------------------------------------|
| **Response** | **Study** | **Component** | **Change per IQR** | **SEM** | **p-value** | **Change per 1 $\mu$g/m$^3$ CI (lower, upper)** |
|-----------------|----------|---------------|-------------------|--------|------------|----------------------------------|
| Heart rate      | 1        | PM$_{2.5}$    | 2.757             | 1.502  | 0.068      | -                                |
|                 |          | EC            | 7.059             | 0.885  | <.0001     | 28.1, 36.1                       |
|                 |          | OC            | -1.845            | 1.431  | 0.199      | -                                |
|                 | 2        | PM$_{2.5}$    | 1.343             | 1.635  | 0.412      | -                                |
|                 |          | EC            | 8.354             | 1.454  | <.0001     | 19.7, 28.1                       |
|                 |          | OC            | 0.879             | 1.107  | 0.428      | -                                |
|                 | 3        | PM$_{2.5}$    | 0.719             | 1.344  | 0.593      | -                                |
|                 |          | EC            | 3.649             | 0.974  | 0.000      | 8.1, 14.1                        |
|                 |          | OC            | -5.489            | 1.543  | 0.000      | -2.4                            |
|                 | 4        | PM$_{2.5}$    | 1.086             | 1.375  | 0.431      | -                                |
|                 |          | EC            | 5.985             | 1.350  | <.0001     | 20.1, 31.9                       |
|                 |          | OC            | 1.916             | 1.266  | 0.131      | -                                |

| lnSDNN          | 1        | PM$_{2.5}$    | 0.094             | 0.026  | 0.000      | 0.0615, 0.0619                   |
|                 |          | EC            | -0.031            | 0.018  | 0.086      | -                                |
|                 |          | OC            | 0.001             | 0.026  | 0.955      | -                                |
|                 | 2        | PM$_{2.5}$    | 0.016             | 0.034  | 0.639      | -                                |
|                 |          | EC            | -0.061            | 0.031  | 0.053      | -                                |
|                 |          | OC            | -0.009            | 0.023  | 0.693      | -                                |
|                 | 3        | PM$_{2.5}$    | 0.024             | 0.029  | 0.415      | -                                |
|                 |          | EC            | 0.006             | 0.020  | 0.774      | -                                |
|                 |          | OC            | -0.038            | 0.032  | 0.240      | -                                |
|                 | 4        | PM$_{2.5}$    | 0.022             | 0.027  | 0.406      | -                                |
|                 |          | EC            | -0.051            | 0.024  | 0.039      | -0.2202, -0.2198                 |
|                 |          | OC            | 0.016             | 0.022  | 0.474      | -                                |

| lnRMSSD         | 1        | PM$_{2.5}$    | 0.001             | 0.017  | 0.942      | -                                |
|                 |          | EC            | -0.048            | 0.011  | <.0001     | -0.267, -0.167                   |
|                 |          | OC            | -0.011            | 0.016  | 0.494      | -                                |
|                 | 2        | PM$_{2.5}$    | 0.019             | 0.016  | 0.236      | -                                |
|                 |          | EC            | -0.023            | 0.015  | 0.127      | -                                |
|                 |          | OC            | 0.007             | 0.011  | 0.541      | -                                |
|                 | 3        | PM$_{2.5}$    | -0.009            | 0.016  | 0.603      | -                                |
|                 |          | EC            | -0.002            | 0.011  | 0.834      | -                                |
|                 |          | OC            | 0.008             | 0.017  | 0.646      | -                                |
|                 | 4        | PM$_{2.5}$    | -0.008            | 0.018  | 0.659      | -                                |
|                 |          | EC            | -0.039            | 0.017  | 0.022      | -0.244, -0.096                   |
|                 |          | OC            | 0.020             | 0.015  | 0.193      | -                                |

Data are expressed as change in heart rate (bpm), lnSDNN (msec) and lnRMSSD (msec), per IQR of pollutant, and per change in 1 $\mu$g/m$^3$ of pollutant.
Table 5 Associations between PM$_{2.5}$, EC and OC and vascular responses

| Response | Study | Component | Change per IQR | SEM | p-value | Change per 1 $\mu$g/m$^3$ CI (lower, upper) |
|----------|-------|-----------|----------------|-----|---------|-------------------------------------------|
|          | 1     | PM$_{2.5}$ | 0.576          | 0.897 | 0.521   | --                                        |
|          |       | EC        | 2.894          | 0.536 | <0.0001 | 10.76, 15.64                              |
|          |       | OC        | -1.555         | 0.824 | 0.061   | --                                        |
|          | 2     | PM$_{2.5}$ | 3.183          | 0.827 | 0.000   | 0.775, 0.779                              |
|          |       | EC        | 2.000          | 0.795 | 0.013   | 3.4, 7.8                                  |
|          |       | OC        | 1.099          | 0.573 | 0.056   | --                                        |
|          | 3     | PM$_{2.5}$ | -0.661         | 0.604 | 0.276   | --                                        |
|          |       | EC        | 0.369          | 0.411 | 0.370   | --                                        |
|          |       | OC        | -0.517         | 0.648 | 0.426   | --                                        |
|          | 4     | PM$_{2.5}$ | -0.377         | 0.555 | 0.498   | --                                        |
|          |       | EC        | 0.893          | 0.521 | 0.088   | --                                        |
|          |       | OC        | 0.876          | 0.473 | 0.065   | --                                        |
|          | Systolic | PM$_{2.5}$ | 0.454          | 0.992 | 0.647   | --                                        |
|          |       | EC        | 3.219          | 0.594 | <0.0001 | 11.9, 17.3                               |
|          |       | OC        | -1.864         | 0.912 | 0.042   | -2.5, -0.88                               |
|          | 2     | PM$_{2.5}$ | 3.392          | 0.903 | 0.000   | 0.825, 0.830                              |
|          |       | EC        | 2.448          | 0.856 | 0.005   | 4.55, 9.45                                |
|          |       | OC        | 1.208          | 0.620 | 0.053   | --                                        |
|          | 3     | PM$_{2.5}$ | -0.268         | 0.673 | 0.690   | --                                        |
|          |       | EC        | 0.986          | 0.453 | 0.031   | 1.6, 4.4                                  |
|          |       | OC        | -1.462         | 0.715 | 0.042   | -1.64, -0.55                              |
|          | 4     | PM$_{2.5}$ | 0.027          | 0.586 | 0.963   | --                                        |
|          |       | EC        | 1.156          | 0.548 | 0.036   | 2.61, 7.38                                |
|          |       | OC        | 0.532          | 0.502 | 0.290   | --                                        |
|          | Diastolic | PM$_{2.5}$ | 0.767          | 0.826 | 0.354   | --                                        |
|          |       | EC        | 2.720          | 0.492 | <0.0001 | 10.16, 14.64                              |
|          |       | OC        | -1.324         | 0.760 | 0.083   | --                                        |
|          | 2     | PM$_{2.5}$ | 3.071          | 0.788 | 0.000   | 0.747, 0.751                              |
|          |       | EC        | 1.979          | 0.766 | 0.010   | 3.51, 7.89                                |
|          |       | OC        | 1.181          | 0.552 | 0.033   | 0.727, 2.07                               |
|          | 3     | PM$_{2.5}$ | -0.772         | 0.584 | 0.187   | --                                        |
|          |       | EC        | 0.258          | 0.398 | 0.517   | --                                        |
|          |       | OC        | -0.097         | 0.628 | 0.878   | --                                        |
|          | 4     | PM$_{2.5}$ | -0.404         | 0.569 | 0.479   | --                                        |
|          |       | EC        | 1.195          | 0.523 | 0.023   | 2.93, 7.47                                |
|          |       | OC        | 1.320          | 0.473 | 0.006   | 1.16, 2.44                                |

Data are expressed as change in mean (MAP), systolic and diastolic pressures (mmHg) per IQR of pollutant, and per change in 1 $\mu$g/m$^3$ of pollutant.

2010. The site is located within 5 km of iron/steel production facilities, a coke oven, oil refinery, sewage sludge waste incinerator, a coal-fired power plant and major highways. Concentrated PM$_{2.5}$ was generated from ambient PM$_{2.5}$ using a Harvard-type fine particle concentrator and whole body animal exposure chambers as previously described in detail [44]. Exposures were carried out in two stainless steel Hinners-type whole body inhalation chambers; one received PM2.5 while the other received HEPA-filtered clean air at the same flow rate as the experimental group. Eight SH rats were exposed in each chamber from 7:30 am – 3:30 pm for four consecutive days (Monday-Thursday). This exposure protocol was repeated on four separate occasions, with four separate cohorts of animals in different weeks: Study 1 (August 10-13, 2009), Study 2 (August 17-20, 2009) Study 3 (July 12 – 15, 2010) and
Study 4 (July 19 -22, 2010). After each 8 h exposure, animals were removed from chambers and returned to their cages.

Exposure characterization
Chamber PM$_{2.5}$ samples were collected on Teflon and Quartz filters (Gelman Sciences, Ann Arbor MI) by attaching Teflon filter packs to the back of the animal exposure chamber with a flow rate of 3 LPM for the duration of each 8-h exposure period. Both ambient and concentrated PM$_{2.5}$ mass concentrations were measured continuously using a Tapered Element Oscillating Microbalance (TEOM). Annular denuder/filter packs were employed to collect major inorganic fine particulate ions. Gravimetric determinations were made using a microbalance (MT-5 Mettler Toledo, Columbus OH) in a temperature/humidity-controlled Class 100 clean laboratory and followed Federal Reference Method (USEPA 1997). PM samples collected on quartz filters were analyzed for carbonaceous aerosols by a thermal-optical analyzer using the NIOSH method (Sunset Labs, Forest Grove, OR). Annular denuder/filter pack samples were analyzed for major ions by ion chromatography (Model ICS-90, DIONEX, Sunnyvale, CA). PM samples collected on Teflon filters were analyzed for a suite of trace elements using inductively coupled plasma-mass spectrometry (ICP-MS) (ELEMENT2, Thermo Finnigan, San Jose, CA).

Cardiovascular telemetry
Two weeks before exposures, animals were surgically implanted with PhysioTel Multiplus transmitters (# C50-PXT; Data Sciences International; DSI, St. Paul, MN) that emit radio signals of electrocardiograms (ECG) and blood pressure (BP). Transmitters were placed with ECG leads terminating in a Lead II configuration to sample cardiac parameters and the pressure catheter placed in the aorta via the femoral artery. Telemetry receivers (RLA3000, DSI) were modified and affixed inside individual cages in exposure chambers that were customized for telemetry studies. Datastreams of 30 second duration were collected and analyzed every 5 minutes during exposures. Automated ECG analysis (DSI, ART3.2) allowed for R-wave detection on a beat-to-beat basis. The R-R intervals for all normal beats (N-N intervals) were used to calculate HR and time-domain measures of HRV: standard deviation of the normal-to-normal intervals (SDNN), an indicator of overall autonomic tone, and the square root of the mean squared differences of successive normal-to-normal intervals (RMSSD), an estimate of parasympathetic tone.

Statistical analyses
To determine exposure-related differences in cardiac and vascular indices we used mixed model analyses using SAS (Version 9.2, Cary, NC); this approach accounts for the longitudinal nature of the measurements on each animal. To reduce the skewness of the HRV measures, we natural log-transformed the SDNN and RMSSD after adding 1. Analyses comparing Air- vs PM$_{2.5}$-exposed rats (Table 2), and the associations between PM$_{2.5}$, EC and OC and cardiovascular responses (Tables 4 and 5) were derived from 1-hour samples of both PM and health effect responses during each of the four field exposure studies. Data for trace elements and carbon subfractions and their associations with health responses (Table 3, Figure 3, Additional file 1: Tables S1 and S2) were derived from 8-hour samples and integrated across the four field exposures (n = 32 samples). The criterion for significance was set at $p \leq 0.05$ for all parameters.
Additional file

Additional file 1: Table S1. Effect of Major Component and Trace Elements on Cardiac Responses. Data are expressed as change in response per IQR of pollutant. PM2.5 components with significant effects are indicated in bold. Table S2. Effect of Major Component and Trace Elements on Vascular Responses. Data are expressed as change in response per IQR of pollutant. PM2.5 components with significant effects are indicated in bold.

Abbreviations
BP: Blood pressure; EC: Elemental carbon; HR: Heart rate; MAP: Mean arterial pressure; OC: Organic carbon; PM2.5: Fine particulate matter; RMSDD: Root mean square of successive differences of adjacent interbeat intervals; SDNN: Standard deviation between normal-to-normal heart beats.

Competing interests
The authors declare that they have no actual or potential competing interests.

Authors’ contributions
JGW developed the study design, carried out the animal experiments and the collection, analysis and interpretation of data, and drafted the manuscript. ASK conducted data analysis and interpretation. MM conducted the collection and characterization of PM2.5 and components, and helped draft the manuscript. JTD oversaw the field site operations, community engagement and helped draft the manuscript. JRG developed the study design and directed field exposures. ACR assisted with study design, data interpretation and helped draft the manuscript. All authors read and approved the final manuscript.

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