Exploration of the Rapid Effects of Personal Fine Particulate Matter Exposure on Hemodynamics and Vascular Function during the Same Day

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List of Abbreviations

BAD, brachial artery diameter

BP, Blood pressure

CV, cardiovascular

DEARS, Detroit Exposure Aerosol Research Study

FMD, flow-mediated dilatation

HR, heart rate

NMD, nitroglycerin-mediated dilatation

PM, particulate matter

$PM_{2.5}$, fine particulate matter < 2.5 μm

PW, pathway

TPE, total personal-level $PM_{2.5}$ exposure

Background: Fine particulate matter < 2.5 μm ($PM_{2.5}$) levels are associated with alterations in arterial hemodynamics and vascular function. However, the characteristics of the same-day exposure-response relationships remain unclear.
Objectives: We aimed to explore the effects of personal $PM_{2.5}$ exposures within the preceding 24 hours on blood pressure (BP), heart rate (HR), brachial artery diameter (BAD), endothelial function (flow-mediated dilatation (FMD)) and nitroglycerin-mediated dilatation (NMD).
Methods: Fifty-one non-smoking subjects had up to 5 consecutive days of 24-hour personal $PM_{2.5}$ monitoring and daily cardiovascular (CV) measurements during summer and/or winter periods. The associations between integrated hour-long total personal $PM_{2.5}$ exposure (TPE) levels (continuous nephelometry among compliant subjects with low secondhand tobacco smoke exposures (n=30)) with the CV outcomes were assessed over a 24-hour period by linear mixed-models.
Results: We observed the strongest associations (and smallest estimation errors) between HR and TPE recorded in the first to tenth hour prior to CV measurements. The associations were not pronounced for the other time lags (11 to 24 hours). The associations between TPE and FMD or BAD did not show as clear a temporal pattern. However, there was some suggestion of a negative association with FMD and a positive association with BAD related to TPE just prior to measurement (0-2 hours).
Conclusions: Brief elevations in ambient TPE levels encountered during routine daily activity were associated with small increases in HR and trends toward conduit arterial vasodilatation and endothelial dysfunction within a few hours of exposure. These responses could reflect acute $PM_{2.5}$-induced autonomic imbalance and may factor in the associated rapid increase in CV risk among susceptible individuals.

Key Words: Sympathetic nervous system, heart rate, endothelium, particulate matter air pollution, personal exposure monitoring

Particulate matter (PM) < 2.5 μm ($PM_{2.5}$) air pollution is associated with an increased risk for cardiovascular (CV) morbidity and mortality, even within the same day of exposure (Peters, et al., 2001). $PM_{2.5}$-induced endothelial dysfunction and elevated blood pressure (BP) may be playing important causative roles (Brook, 2008). Indeed, higher levels of ambient $PM_{2.5}$ have been associated with blunted brachial flow-mediated dilatation (FMD) and elevated BP (Auchincloss, et al., 2008; Chuang et al., 2005; Dales, et al., 2007; Dvonch, et al., 2009; Ebelt et al., 2005; Langrish, et al., 2009; Liu et al., 2009; O'Neill, et al., 2005; Rundell, et al., 2007; Schneider, et al., 2008; Zanobetti A, et al., 2004). However, most studies have only examined these outcomes 1 or more days following relatively prolonged exposures (e.g. 24 to 72 hour mean $PM_{2.5}$ levels) (Brook and Rajagopalan, 2009). Whether brief (e.g. hour-long) exposures to $PM_{2.5}$ at commonly encountered present-day ambient levels are also capable of altering arterial hemodynamics and vascular function as rapidly as within the same day and the characteristics (e.g. magnitude, consistency) of any such responses remain unclear. As 2 hour-long exposure to very high particle levels under experimental conditions can elevate BP and impair FMD within hours (Brook, et al. 2009), it is plausible that $PM_{2.5}$ at lower ambient levels may induce similar responses.

The relationship between personal exposures and ambient $PM_{2.5}$ mass concentrations can significantly vary among different individuals with personal levels potentially being more predictive of health responses (Rodes et al., 2010; Brook et al, 2010). Exposure characterizations at the personal-level thus advance our understanding of the complex relationships between pollution exposures and diseases (Weis et al., 2005). The CV sub-study of the Detroit Exposure and Aerosol Research Study (DEARS) was designed to elucidate the air pollution components and time frames of exposure responsible for affecting several CV outcomes including arterial hemodynamics (i.e. BP and heart rate (HR)) and vascular function. The initial results compared the effects associated with daily (24-hr) mean ambient $PM_{2.5}$ exposures assessed at the personal versus community levels (Brook et al., 2010). In this analysis, we aimed to gain insight into the acute same-day exposure-response relationships at a finer time scale resolution. As such, we explored the associations between the CV outcomes, including BP, FMD, and HR, with changes in total personal $PM_{2.5}$ exposure (TPE) measured each hour during routine daily activity throughout the preceding 24-hour period.

Materials and Methods

This study was approved by the Institutional Review Boards of the University of Michigan and RTI International along with the Human Subjects Approving Official of the U.S. Environmental Protection Agency (EPA). The main DEARS protocol has been previously described in more details elsewhere (Williams, 2005; Williams et al., 2009). All participants were non-smokers living in a non-smoking household, at least 18 years old, ambulatory, and capable of understanding the consent documentation. There were no exclusion criteria for race, occupation, sex, medications, or health status. In addition to community and residential $PM_{2.5}$ site monitoring that occurred within 6 Detroit area neighborhoods during summer and winter periods over 3 years (6 total seasons), TPE was also measured for 5 consecutive days during winter and/or summer months using personal vest monitors in one study volunteer from each participating household. DEARS exposure study participants were invited without any restrictions to also participate in the CV sub-study while it was occurring (during seasons 2-6). Volunteers crossing over into the CV study underwent an additional visit at which time written informed consent was obtained and the average of the 2nd and 3rd of three seated BP measurements was determined along with a fasting lipid profile and glucose (Cholestech LDX analyzer, Cholestech Corp).

Exposure Assessments

The primary $PM_{2.5}$ personal monitoring being reported here was performed using a modified personal DataRam nephelometer (pDR-1200, Thermo Electron, Franklin, MA). These devices are capable of the near-continuous active sampling and analysis of PM in either an active or passive sampling mode. The device was operated in an active mode (30 second data collection intervals) in the DEARS so that a true size fractionation of airborne PM aerosol could be estimated over the course of each 24-hr monitoring period. Modification of the basic unit consisted of installation of an active $PM_{2.5}$ Personal Environmental Monitor (PEM) size fractionating inlet upstream of the optical chamber. Williams et al. (2009) have previously described in detail the use and modification of this monitor in the DEARS. The basic personal nephelometer deployed here has been used to provide thousands of short interval PM mass concentration measurements in human exposure longitudinal panel studies. We have previously reported upon the operation and utility of the monitor for such purposes (Wallace et al., 2006) and their general comparability to filter-based (gravimetric) measurements (Rea et al., 2001). He et al.

(2010) have recently reported the successful use of a similar version of this personal nephelometer in characterizing $PM_{2.5}$ influence of HR variability alterations in an adult cohort.

The personal nephelometer was one of a number of monitoring devices incorporated into an exposure monitoring vest that each study participant wore throughout each day. $PM_{2.5}$ nephelometry was initiated on each monitoring day (Tuesday through Saturday) at a consistent time (9 am ± 2.5 hr). Each monitoring session represented a continuous 24 hour period of air collection using a lightweight nylon monitoring vest to secure the sampler's inlet in the breathing zone of the participant (Williams et al., 2003; Williams et al., 2009). Participants were instructed to wear the vest at all times except for periods of sleeping, bathing, and the changing of clothes, at which times the monitor was directed to be kept as close as possible to the subject (e.g. next to the bed at night). Quality of the nephelometric data collected in the study was ensured through rigorous QA/QC procedures. In brief, each nephelometer was chamber calibrated immediately prior to each DEARS monitoring season using a challenge aerosol of $PM_{2.5}$ size fractionated ammonium nitrate which was known to make up a sizeable component of the Detroit airshed. In addition, each unit was audited once during the season for any response changes in in its calibration as well as at the end of the seven week study period. Daily audits of monitor flow rate, battery condition among other parameters were performed. Audits of the units zero concentration response point (baseline) were performed using a HEPA-grade airstream. Study data were reviewed each day for completeness (length of successful monitor operation), impact of relative humidity (RH) on monitor response, and overall data acceptability criteria (monitor response relative to changes in minute by minute data values). Wallace et al. (2006) have described in detail the procedures used here to determine overall data acceptance criteria. Because nephelometric response is known to be RH sensitive, continuous RH measurements were taken simultaneously (Williams et al., 2009). These RH were then used to establish an algorithm to correct nephelometric response for such bias. A full description of this algorithm will be reported elsewhere. All data reported here were RH-corrected.

The monitoring vest deployed here contained continuous sensors that monitored how compliant participants were with wearing it during the non-exclusion scenarios or time of day (e.g. sleeping, bathing). These sensors collected information on physical activity levels and body temperature among other parameters of the participant. These

electronic data were then cross-checked with 15-minute interval time activity diaries completed each day by the participant to note periods of reported sleeping, bathing, or performing other activities resulting in the monitoring equipment not being worn. Findings from this review resulted in a level of general monitoring compliance for each monitoring period. Only monitoring data from participants meeting a pre-specified conservative compliance rate of 60% were analyzed in this study, for reasons described elsewhere (Williams, et al, 2009; Rodes et al., 2010).

While all participants recruited into the DEARS were self-reported to be non-smokers living in non-smoking households, each participant's exposure to secondhand smoke (SHS) was also measured simultaneously with the nephelometric monitoring. These data were collected using another collocated $PM_{2.5}$ PEM inlet also affixed to the monitoring vest. This inlet contained a 37 mm Teflon filter (Teflo, Gelman Science, Ann Arbor, MI) for active (2 lpm) exposure monitoring. The subsequent filter samples were optically analyzed for a mass-based estimate of SHS using a technique previously described (Lawless et al. ,2004; Williams et al.,2009; Rodes et al., 2010). Only the results from subjects with a pre-determined rate of less than 1.5 $\mu g/m^3$ of mean daily SHS exposure were included in this analysis to avoid its potential confounding effect on the CV outcomes as described elsewhere (Brook et al., 2010). Rodes, et al. (2010) has provided a detailed examination of potential SHS impact upon personal exposures in the DEARS and the threshold of 1.5 $\mu g/m^3$ being employed to note such influence. It should be noted that the SHS threshold value being used here results in a very conservative data inclusion criteria.

The filter sample described above also provided the means to establish a filter-based gravimetric estimate of personal $PM_{2.5}$ exposure that could be compared with the continuous nephelometric response. This filter-based sampling technique and the actual monitoring device used here has been used to collected thousands of personal exposure samples. It provided for a 24-hr integrated estimate of $PM_{2.5}$ personal exposure in the DEARS as described elsewhere (Williams et al., 2009; Brook et al., 2010; Rodes et al., 2010). Data from collocated personal monitoring of both samplers from each personal monitoring event were then used post-study to determine the level of agreement between the two techniques (nephelometry and gravimetric). Nephelometric mass concentrations ($\mu g/m^3$) were highly correlated with those from the filter-based sampler. Integration of the personal nephelometric data from each 24-hr monitoring period to provide a single average concentration which in turn was compared with the filter-based

monitoring resulted in a mean coefficient of determination of R^2= 0.80 (range of 0.75 to 0.85) over the three DEARS monitoring periods (seasons 4-6) being reported here. This level of agreement was believed to be indicative of the high degree of QA/QC that was employed for the study, the use of the same size fractioning inlet for both monitoring types, the use of a calibration aerosol (ammonium nitrate) similar to that encountered in Detroit, and correcting for RH bias among other factors.

It should be mentioned that all of the $PM_{2.5}$ nephelometric data being reported here represents total personal $PM_{2.5}$ exposures (TPE). No source categorization of the aerosol into its various components (PM of ambient origin, PM of non-ambient origin, etc.) is possible at this time. Non-ambient sources would potentially include cooking aerosols, mobile source emissions among others. Such sources often comprise 50% or more of the TPE (Wallace et al., 2006). Future efforts will attempt to integrate observational survey data collected in the DEARS and determine individual sources of short duration impacting the nehelometric response.

Cardiovascular Endpoints

CV study visits were performed at the participant's home for up to 5 consecutive evenings, Tuesday through Saturday, between 4 and 7 PM. These visits took place on concurrent days while subjects wore the vest monitors. There were 6 CV outcomes: systolic and diastolic BP, HR, brachial arterial diameter (BAD) (indicative of basal arterial tone), FMD and nitroglycerin-mediated dilatation (NMD) (indicative of smooth muscle function). Participants were instructed to maintain their daily routine, including taking all medications, but to fast for at least 4 hours prior to the scheduled visits and to avoid unusual physical activity. During each visit, subjects rested supine for 10 minutes prior to automated BP and HR measurement (Omron 780 monitor) that was obtained in triplicate with a one minute lapse between measures. The average of the 2nd and 3rd BP and HR recordings was used for analyses (Pickering, et al., 2008).

BAD and FMD were next determined. Brachial images were obtained with a portable Terason2000 ultrasound system with a 10.0 mHz linear array transducer with ECG-gated image acquisition (http://www.terason.com/; Teratech, Corp.). Five minutes of upper arm occlusion using a rapidly deflating arm cuff was performed in order to determine FMD, which was defined as the mean percent increase in BAD above baseline diameter from between 50-70 seconds after cuff deflation. Images were analyzed using semi-automated edge detection software (Vascular Research Tools,

Medical Imaging Applications; http://www.mia-llc.com/). NMD was next determined as the percent dilatation of the BAD 3 minutes following 0.4 mg of sublingual nitroglycerin. Detailed descriptions of the methods have been previously described and accord with guidelines (Brook, et al. 2005), while the reproducibility of our testing is reported elsewhere (Brook, et al., 2009). As compared to the brachial FMD study technique performed in our controlled laboratory setting as described elsewhere, the only substantive methodological differences in this study are the lack of room temperature standardization and the fact that subjects fasted for only at least 4 hours prior to measurements.

Statistical Assumptions and Models

Integrated hour-long total $PM_{2.5}$ exposures (i.e., TPE) during the preceding 24 hour period, calculated for each individual starting immediately before the time of their CV outcome measurements, were determined from the vest continuous nepholometry data (i.e. 24 individual hour-long periods from lag 0-23 hours), with lag 0 representing the time between 0-60 minutes before the CV outcome measurement. Two subjects with 3 observation-periods were removed from analyses because >25% of their hourly TPE levels during a day were negative (i.e. <0 $\mu g/m^3$), indicative of a systematic error in the vest monitoring system on that day per study protocol. The associations for each hour-long TPE period were made for 6 pre-specified outcome variables: systolic BP, diastolic BP, HR, BAD, FMD, and NMD. All of these CV outcomes were observed for 5 consecutive days for each season (winter versus summer) within a subject.

The subjects were considered to be selected at random from a population of pre-selected neighborhoods per the design and methods of the main DEARS cohort as described in more detail elsewhere (Williams, 2005; Williams et al., 2009). Considering the possibility that within-subject errors were auto-correlated, a linear mixed model was employed since it was more appropriate when data were collected over time on the same subjects (Brady, et al. 2007; Mohamed, et al., 2007). We thus assumed that the association between each of the responses evaluated and exposure to TPE is linear with an intercept varying at random over individuals but a slope was assumed to be the same for all subjects. Several predictors of the response were included in the model as fixed effects: age, gender, race, body mass index (*BMI*) and ambient temperature (*Temp*). The relationship between these predictors and responses was assumed to be common

to all subjects. In equation (1),

$$Y_{ij} = \beta_0 + \beta_1 h_k.PM_{2.5} + \beta_2 age + \beta_3 gender + \beta_4 race + \beta_5 BMI + \beta_6 Temp + \alpha_{i`} + \varepsilon_{ij}$$

where Y_{ij} is the response, a CV outcome for subject *i* at study day *j*, $h_k.PM_{2.5}$ the k-hour lag TPE prior to the health outcome measurement time, and $Temp$ the ambient community-level temperature. This model (1) includes fixed effects associated with the subject-level covariates (β's), a random effect associated with the intercept for each subject (α_i) and a residual associated with each observation (ε_{ij}). The random effects by subject were assumed to be independently distributed across subjects with a normal distribution $\alpha_i \sim N(0, \delta^2)$. The within-subjects errors ε_{ij} were assumed to be distributed $\varepsilon_{ij} \sim N(0, \sigma^2 R_i)$, where R_i was the variance-covariance matrix for the residuals. It was also assumed α_i and ε_{ij} were independent of each other.

The first-order autoregressive structure, denoted by AR(1), was explored for the covariance R_i in the analysis, which implies observations closer to each other in time exhibit higher correlation than observations farther apart in time. The likelihood ratio test showed the AR(1) correlation structure did not improve the fit. Other available covariates including season (i.e. winter versus summer), neighborhood indictor, and the subject's study day (e.g. first versus second day of monitoring during the 5 day period) were not included in the final model as they did not predict responses individually or alter the significance of any results. We could only examine a limited number of time periods simultaneously due to the relatively few number of repeated responses per subject (maximum of five per season). Complex distributed time lag models beyond those incorporating a few lagging periods were therefore not analyzed and also because of highly correlated dependent and independent variables were inappropriate models. Whilst the effects of several different moving averages of exposure duration were explored, a complete description of the time course of personal $PM_{2.5}$ exposure was most thoroughly examined for the purposes of this exploratory analysis by using 24 multiple models, each with a single hourly time lag exposure measure. The analysis was performed by function "lme (linear mixed-effects model)" in R (version 2.8.1). Statistical significance was defined as $p<0.05$.

Results

The characteristics of the total cohort (n=51) of subjects enrolled during seasons 4-6 who had both the CV outcomes performed and TPE measured by continuous nephelometry are presented in Supplement Tables 1 and 2. Twenty-six subjects (51%) had no self-reported CV disease or risk factor. Ten subjects participated in 2 separate consecutive seasons; thus, there were a total of 61 subject-observation periods. As subjects could contribute up to 5 consecutive days of data during each season, there were a total of 265 observation-days. Seventy-four (28%) and 91 (34%) observations were excluded by the vest compliance and low SHS rule, respectively. There were 38 subjects (191 observations) who met the 60% vest compliance rule only and 30 (102 observations) who also met the low SHS rule (vest-low SHS sub-group). Table 1 demonstrates the characteristics of the vest-low SHS sub-group from which the main outcomes of this study are derived. The results from the vest compliant group were similar to those from the vest-low SHS sub-group for the BAD and FMD outcomes (2 hour lag time) but not for HR changes (less significant results). Therefore, due to potential confounding effects of SHS, the main results (per methods section) are provided for the vest-low SHS group (Table 1).

The change in the CV parameters per 10 $\mu g/m^3$ of TPE are presented by lagging time in hours (0 to 23) in Figures 1 (HR), 2 (BAD), and 3 (FMD) in addition to the 95% confidence intervals. It is difficult to interpret the true statistical significance of each time lag due to the many lags examined. However, we did observe some tendencies in the temporal pattern. Positive associations were observed for time lags of 1-10 hours for HR (+0.38 to +0.78 beats/min per 10 $\mu g/m^3$) while no such association was evident for the 11-23 hour lags. The 1-10 hour time lag effects were also estimated with more precision (i.e. narrower confidence intervals) compared to the longer time lags. The temporal pattern between TPE and either BAD and FMD was neither as clear nor consistent. However, there was an overall trend for positive and negative associations for BAD and FMD, respectively, during the 24 hour period. The strongest evidence for an association was at the 2 hour time lag for both FMD (negative association) and BAD (positive association). No consistent relationships with TPE (equal numbers of positive and negative associations) were observed for the BP levels or NMD (Supplemental Figures 1-3).

Adding additional available variables to the model (e.g. season, visit day) did not affect the results. Whilst the general trends remained similar, evaluating the associations with longer moving averages (2-6 hours) did not provide further insights into the nature

of the TPE-response relationships beyond the more complete information gained by examining the effect of each hour individually (results not shown). In this context, the mean TPE integrated over the entire preceding 24 hour period was significantly associated only with an increase in HR (0.78 beats/minute per 10 μg/m^3, $p<0.05$). We also examined the interaction effects between TPE and medications in 2 ways: usage of a beta blocker and usage of any of the 5 CV medications reported (Supplement Table 2). As only 3 subjects in the vest-low SHS sub-group were using a beta blocker, we did not evaluate the effects in these subjects alone. On the other hand, the association between TPE and CV outcomes remained almost the same with no changes in significance after excluding these 3 subjects. Six subjects were using any medication. The medication interaction effects for HR, BAD and FMD were insignificant, and thus the main association between TPE and the 3 CV outcomes were not changed in significance. Finally, there were no consistent findings related to subjects' health status (body mass index, initial HR or BP, age) modifying the outcome associations. Low CV risk subjects (Framingham risk score below the mean) had similar positive HR associations as the total cohort (56 observations). Fewer subjects (27 observations) had above the mean risk and thus the associations were not significant. The results are of unclear validity given the limited sample size, but do not generally suggest that higher risk patients have larger CV responses (or are principally responsible for the findings).

Discussion

$PM_{2.5}$ exposure has been shown capable of increasing BP/HR and impairing vascular function. However, these responses have typically been observed to occur 1 or more days following exposures that are ≥ 24 hours in duration and as estimated by ambient community levels (Brook and Rajagopalan, 2009). This is the first study to report the BP and HR changes together with the vascular responses associated with brief personal $PM_{2.5}$ exposures as they change each hour throughout the preceding day during routine activity. Higher TPE levels (without source considerations) encountered during several periods (most consistently during the most recent 11 hours) were related to small increases in HR. In addition, there were concomitant early (lag hour 2) trends toward conduit artery vasodilatation (increased BAD) and impairment in endothelial function (decreased FMD). Whilst these effects were small in size and intermittently statistically significant, they did occur in a consistent and coherent biological manner. We acknowledge that this study was exploratory and should be considered hypothesis-

generating. While accepting that chance statistical associations cannot be excluded given the numerous observations evaluated and that the health significance must remain speculative, these responses (along with the underlying physiological pathway likely responsible) could help to explain the mechanism underlying $PM_{2.5}$-mediated CV events that occur during the same day of exposure among susceptible individuals (Peters, et al., 2001).

Few studies have investigated the effects of PM exposures within the same day on BP, HR, or vascular function (Chuang, et al., 2005; Dales, et al., 2007; Langrish, et al., 2009; Rundell, et al., 2007; He, et al., 2010). In the most similar previous study, both BP and HR increased in relation to higher personal ultrafine particle levels encountered 1-2 hours earlier among 10 patients with lung disease (Chuang, et al., 2005). However, the investigators did not examine vascular function, periods of exposure prior to 4 hours, or the effects of $PM_{2.5}$. The study was small and restricted to patients with lung disease; therefore, its pertinence to the general population is questionable. Four other studies have investigated the acute effect of brief ambient estimates of $PM_{2.5}$ exposures (30-120 minutes) within the same day on similar CV parameters. However, in each report, except for that of He, et al. (discussed later), exposures were generated by artificial scenarios that conveyed higher concentrations than routinely encountered and with the responses evaluated only once within minutes thereafter. PM has been shown to impair FMD without affecting BP or HR at a bus stop ($PM_{2.5}$ ~40 $\mu g/m^3$) (Dales, et al., 2007), to raise BP with a trend toward an increase in HR while walking next to city streets in Beijing ($PM_{2.5}$ ~86-140 $\mu g/m^3$) (Langrish, et al., 2009), and to cause a decrease in BAD and FMD immediately after exercising close to a roadway (ultrafine counts ~115,000-134,000 particles/cm^3) (Rundell, et al., 2007). Despite variations in specific findings (possibly due to multiple methodology differences), these studies generally support our findings and the hypothesis that PM could pose an immediate threat to the CV system within hours of exposure. Our results significantly extend these reports by more fully characterizing the temporal relationships between several concomitantly measured CV outcomes with brief personal-level $PM_{2.5}$ exposures encountered throughout the preceding full 24 hour period at more typical present-day ambient levels. Two other studies corroborate our finding that a more prolonged exposure (previous 24 hour-long mean personal $PM_{2.5}$ level) can cause a small increase HR around 0.44 to 1.15 beats/minute (Mar, et al., 2005; Liu, et al., 2009).

In contrast to HR, we did not observe a coherent effect of same-day TPE levels on BP. This differs from some of our previous findings. Higher community $PM_{2.5}$ levels measured 2-5 days earlier in 3 Detroit areas have been associated with elevations in BP (Dvonch, et al., 2009). Integrated 24-hour TPE levels measured by filter-based methods from subjects participating in this CV sub-study of DEARS averaged from approximately 8-32 hours prior to the time of BP measurement were also associated with an increase in systolic BP (1.4 mm Hg per 10 $\mu g/m^3$) (Brook, et al. 2010). Two other previous studies have reported a same-day effect of comparatively higher levels of particle exposures upon BP (Chuang, et al., 2005; Langrish, et al. 2009) while another did not (Dales, et al., 2007). Differences in pollutant concentrations, characteristics, constituents, or subject susceptibilities may have been responsible for these discordant findings among studies. However, it is important to note that the BP elevations we twice previously observed in relation to ambient $PM_{2.5}$ levels in the same region (Detroit communities) only occurred in a delayed fashion. We hypothesize that given the multitude of physiological parameters regulating systemic arterial BP that these relatively low TPE concentrations may require a longer cumulative duration of exposure and/or lag period in order to elicit an observable effect (Brook and Rajagopalan, 2009). On the other hand, the biologic pathway responsible for causing an increase in HR may be more acutely sensitive to these low-level $PM_{2.5}$ concentrations or alternatively small changes in chronotropic responses may be a more statistically discernible outcome.

Potential Biological Mechanisms

This study was not designed to elucidate the mechanisms responsible for the observed CV changes. We acknowledge that any such discussion is speculative. However, we believe the sum results can be interpreted to provide an overall hypothesis. There are 3 general pathways including systemic inflammation (pathway 1), altered autonomic nervous system balance (pathway 2), and direct effects of particles or constituents reaching the circulation (pathway 3) that could link $PM_{2.5}$ exposure with changes in CV physiology (Brook, 2008). It cannot be excluded that each pathway alone and/or as an integrated response all-together were responsible for the seemingly mixed physiological changes observed in HR, FMD, and BAD. However, given the rapidity of the responses it is probable that pathways 2 or 3 were chiefly involved. Though effects via pathway 3 cannot be excluded, given the low concentrations of $PM_{2.5}$ it seems most plausible to principally instigate pathway 2. Thus, we believe that acute

vagal withdrawal with a (relative) increase in sympathetic nervous system activity likely caused the elevation in HR.

It is also possible that pathway 2 alone could have caused the observed trends towards conduit artery vasodilatation and blunted endothelial function within this rapid time frame. There is complex interaction between the autonomic nervous system and vascular tone/endothelial function (Harris and Matthews, 2004). Acute sympathetically-mediated α-receptor-induced vasoconstriction in the resistance arterioles is regionally discordant, occurring in mesenteric but not muscle beds (i.e. brachial artery) upon sympathetic stimulation. In addition, a vasoconstrictive response is blunted by a reflex increase in basal nitric oxide release in conduit vessels supplying skeletal muscles and β_2 receptor activation that cause vasodilatation (i.e. increased BAD) allowing for adequate muscle blood flow under periods of stress. Other studies also demonstrate that sympathetic activation subsequently leads to a rapid impairment within hours in stimulated (e.g. flow-mediated) brachial artery endothelial-dependent vasodilatation (Hijmering, et al., 2002). Thus, all 3 outcomes observed in this study may have stemmed from a rapid imbalance of autonomic nervous system activity. A recent study supports this speculation (He, et al. 2010). The authors similarly found that HR increased rapidly 3-6 hours following elevations in personal-level $PM_{2.5}$ exposure. Moreover, HR variability metrics suggestive of autonomic imbalance favoring sympathetic tone occurred during this same rapid time window.

Strengths and Limitations

The CV sub-study of DEARS is the first to investigate the effect of air pollutants on these CV outcomes measured in the actual "field" (i.e. households of subjects). This methodology avoided the atypical exposures and activities that would occur when subjects travel to a research laboratory and thereby strengthened our ability to observe the "real" exposure-response associations occurring on typical days. Conducting personal-level $PM_{2.5}$ exposure assessments and adjusting for the confounding effects of vest compliance and SHS also minimized the level of exposure misclassification (Rodes et al., 2010). Such steps also improved the data quality and the proper characterization of those most-exposed at the individual level, which in turn improved the power of the data content at all levels of the exposure distributions. These procedures strengthened our capabilities to observe small health effects, as reported earlier (Brook, et al., 2010) within a relatively small sample size and theoretically added support for the veracity of

the health-exposure associations. These robust methodologies are critically-important in order to establish exposure-to-outcome linkages, particularly given that the composition and character of the $PM_{2.5}$ can differ across the exposure distribution (Edwards and Jantunen, 2009).

We also recognize several limitations. No attempt was made to examine the personal exposure factors or time activity patterns of the participants and their potential impact upon the health outcomes. Future investigations are planned that will determine the ambient and major non-ambient sources encountered by participants in various locations as well as their impact upon significant health outcomes. The study was also not designed to assess the associations between hourly TPE levels and the CV outcomes. The exploratory nature and multiple comparisons performed in this analysis make it possible that some of the significant results represent chance findings. However, we believe that the consistent effects on HR within the first 11 hours and the coherency of the trends of effects for BAD and FMD throughout the monitoring period suggest that true biological responses are likely occurring. It is possible that having more observations would have provided additional power and many of the borderline non-significant time points in the BAD and FMD trends would have become statistically significant. Future appropriately designed and powered studies are required to corroborate these novel findings. The ultrasound measurements were also performed at the subject's household. Whilst standardized methodologies and subject preparation were carefully followed (Brook, et al., 2005) the reproducibility of the FMD and BAD may have been less than what can be obtained in a vascular laboratory. However, we believe that the overall strengths of this design (i.e. performing the studies in the actual "field") outweighed this limitation. The statistical significance of the small changes observed for HR, BAD, and FMD suggest that the sample size using these methods was adequate. Finally in regards to the vascular responses, there was a trend towards an increase in the basal BAD during the time period analyzed. A larger initial BAD could mathematically produce a smaller FMD response when the latter is expressed as a percent dilatation from baseline. We cannot exclude that this may have contributed to a decrease in FMD (%) and future studies should therefore analyze both the percent and absolute (in mm) vasodilatory responses when basal diameter (i.e. resting arterial tone) may be altered.

Though the main DEARS protocol sampled a random representation of the local residents, the findings from this sub-study may not generalize to the entire population. However, as compared to previous similar studies, the participants in this analysis

represented a relatively larger cohort of individuals with a broader range of health conditions. Whilst we performed exploratory analyses regarding potential effect modifiers (e.g. medications, demographics), further investigation related to the effects of patient susceptibilities is warranted. Finally, the responsible $PM_{2.5}$ components and sources could not be determined from these data. We also did not have hourly personal-level gaseous pollutant information and there is a possibility for unrecognized additional, or confounding, effects of these co-pollutants. On-going and planned analyses of may help provide future insights into these important matters.

Conclusions

Higher TPE levels encountered during routine daily activity were related to small increases in HR and trends toward endothelial dysfunction within hours of exposure among individuals living in several Detroit-area communities. Though the clinical relevance of these responses remains unknown, the findings support in general the notion that present-day levels of $PM_{2.5}$ could potentially rapidly affect CV physiology in a manner contributing to the instigation of acute CV events in susceptible people.

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Table 1 Subject Characteristics in the vest compliance and low SHS sub-group (n=30)

FACTOR	Number of observations	MEAN or %	SD	Minimum	Q1*	Median	Q3*	Maximum
Age (years)	30	45.4	14.3	22	33	46	54	73
Sex	30							
Female	25	83%						
Male	5	17%						
Race	30							
African American	13	43%						
Caucasian	16	53%						
American Indian	1	3%						
Body mass index (Kg/m^2)	30	29.9	5.9	21.8	25.3	29.9	33.0	48.2
SBP (mm Hg)	100	123.0	16.5	91	110	124	136	167
DBP (mm Hg)	100	73.5	10.4	53	65	73	81	101
HR (beats/min)	100	74.0	10.2	51	67	74	79	100
BAD (mm)	98	4.0	0.9	2.5	3.4	3.9	4.6	6.2
FMD (%)	93	4.0	5.2	-6.0	0.4	2.9	6.9	18.3
NMD (%)	47	15.2	7.0	1.5	9.9	15.4	19.0	31.9
Average of 24 hourly $PM_{2.5}$ ($\mu g/m^3$)	98	32.1	26.2	2.5	13.1	24.3	43.1	122.2
Daily average of personal $PM_{2.5}$ ($\mu g/m^3$)	102	18.0	10.4	1.3	9.9	15.8	23.4	51.9
Daily average of ambient $PM_{2.5}$ ($\mu g/m^3$)	97	15.8	7.6	5.9	9.9	13.0	20.4	38.9

SHS, secondhand smoke; SD, Standard deviation; SBP, systolic blood pressure (BP); DBP, diastolic BP; HR, heart rate; BAD, brachial artery diameter; FMD, flow-mediated dilatation; NMD, nitroglycerin-mediated dilatation.

*Q1, 25^{th} percentile; Q3, 75^{th} percentile

Figure 1 Personal $PM_{2.5}$ and Heart Rate (HR)

The associations of hourly TPE levels with HR change according to the lag period of exposure. For example, hour 0 = period from 0-60 minutes prior to the CV measurement. Points equal the multivariate adjusted HR association (β coefficient per 10 μg/m^3 increase in TPE ± 95% confidence intervals) for each hourly time point from the linear mixed model (1). Statistically significant time points are bolded in square shape for $p<0.01$ and in diamond shape for $p<0.05$. The x-axis is the period of time (hour lag) prior to the measurement of the CV outcome.

Figure 2 Personal $PM_{2.5}$ and Brachial Arterial Diameter (BAD)

The associations of hourly TPE levels with BAD changes according to the lag period of exposure. For example, hour 0 = period from 0-60 minutes prior to the CV measurement. Points equal the multivariate adjusted BAD association (β coefficient per 10 μg/m^3 increase in TPE ± 95% confidence intervals) for each hourly time point from the linear mixed model (1). Statistically significant time points are bolded in square shape for $p<0.01$ and in diamond shape for $p<0.05$. The x-axis is the period of time (hour lag) prior to the measurement of the CV outcome.

Figure 3 Personal $PM_{2.5}$ and Flow-Mediated Dilatation (FMD)

The associations of hourly TPE levels with FMD changes according to the lag period of exposure. For example, hour 0 = period from 0-60 minutes prior to the CV measurement. Points equal the multivariate adjusted FMD association (β coefficient per 10 μg/m^3 increase in TPE ± 95% confidence intervals) for each hourly time point from the linear mixed model (1). Statistically significant time points are bolded in square shape for $p<0.01$ and in diamond shape for $p<0.05$. The x-axis is the period of time (hour lag) prior to the measurement of the CV outcome.

Figure 1

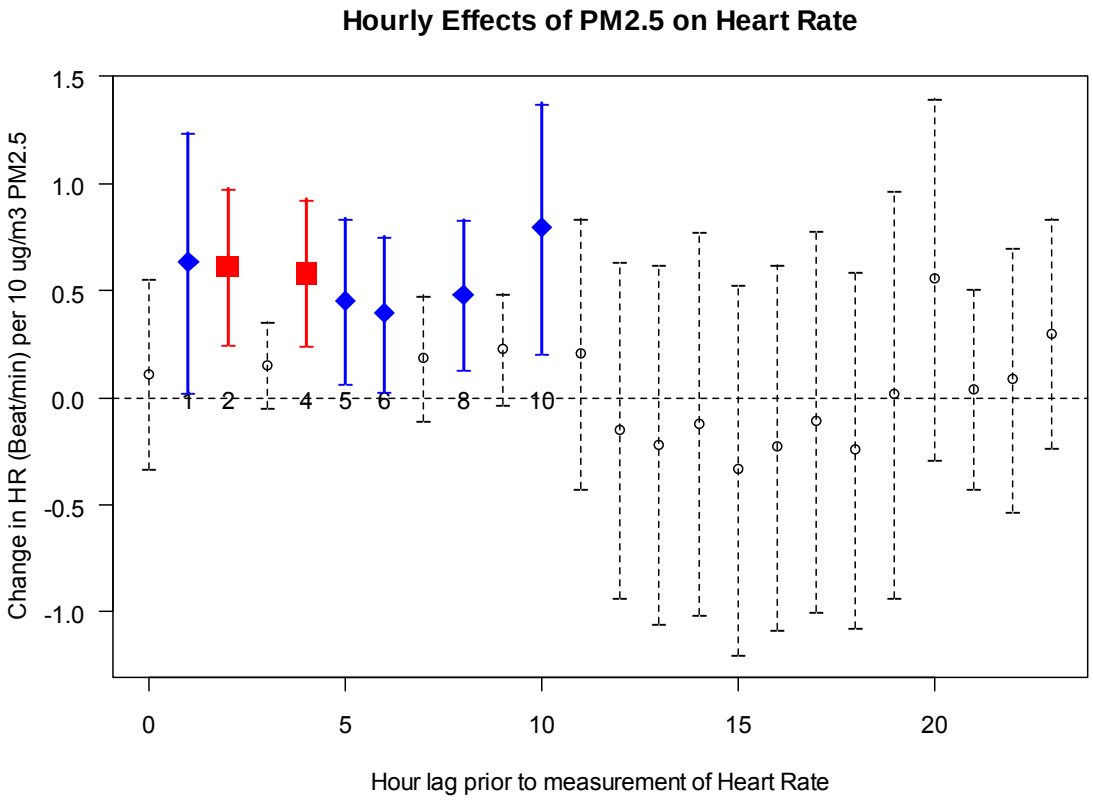

Hourly Effects of PM2.5 on Heart Rate
Change in HR (Beat/min) per 10 ug/m3 PM2.5
1.5
1.0
0.5
0.0
-0.5
-1.0
1
2
4
5
6
8
10
0
5
10
15
20
Hour lag prior to measurement of Heart Rate

Figure 2

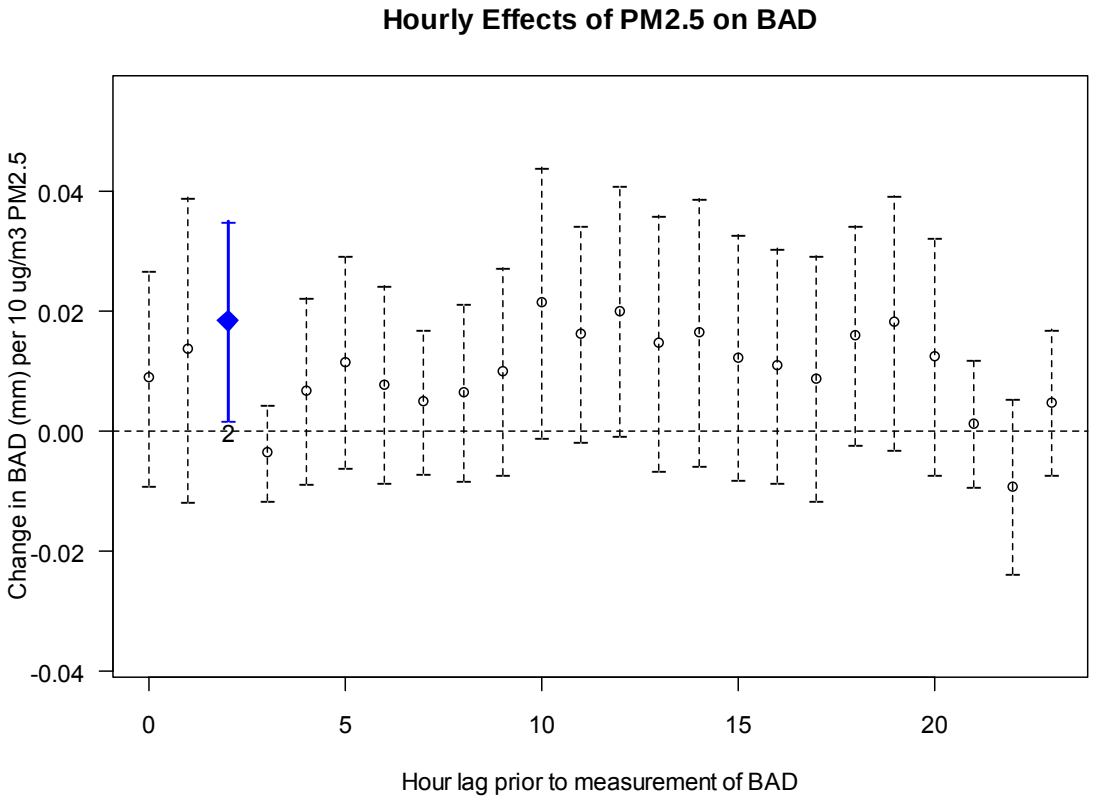
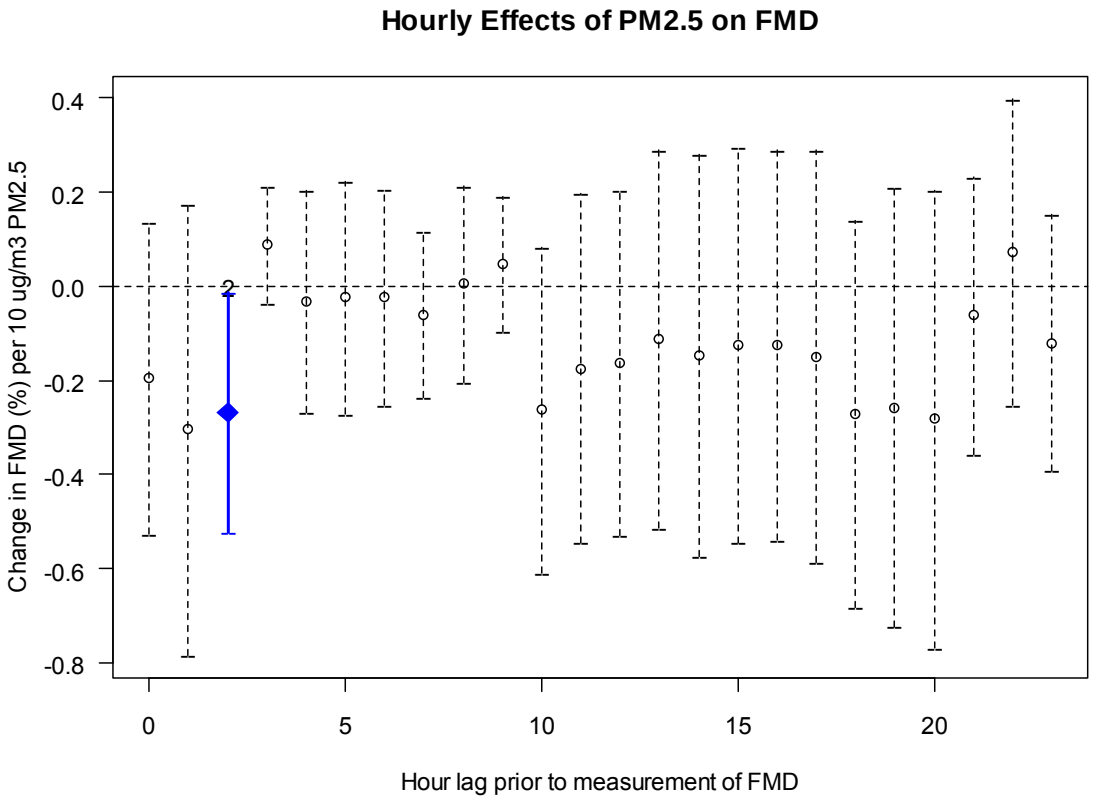

Hourly Effects of PM2.5 on BAD
Change in BAD (mm) per 10 ug/m3 PM2.5
0.04
0.02
0.00
-0.02
-0.04
2
0
5
10
15
20
Hour lag prior to measurement of BAD

Figure 3


Hourly Effects of PM2.5 on FMD
Change in FMD (%) per 10 ug/m3 PM2.5
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
0
5
10
15
20
Hour lag prior to measurement of FMD