

## ABSTRACT

Since 1997, epidemiological and animal studies have identified many potential mechanisms by which particles may impact health. However, the relative importance of these potential pathways and the steps along these pathways are not well understood, particularly as to how they relate to specific particle components and sources, for which pathways are likely to differ. In our original EPA-funded Particle Center, we examined ways in which we could use epidemiological studies to further our understanding of the mechanisms of particle toxicity. As part of this effort, we studied the air pollution mediated response of individuals participating in the Normative Aging Study (NAS), a large prospective cohort living in Eastern Massachusetts. We collected electrocardiograms (ECGs) and blood samples from each study participant and analyzed these samples for heart rate variability (HRV) and C-reactive protein (CRP), respectively. We found ambient PM<sub>2.5</sub> and black carbon (BC) concentrations to be associated with decrements in HRV. Ambient BC concentrations were further found to be associated with increased CRP and fibrinogen levels. These results suggest that the particle mediated autonomic changes may be brought about through pathways involving both the autonomic nervous system and systemic inflammation. Definitive identification of particle-mediated biological mechanisms was limited, however, by the lack of other intermediate cardiac and inflammation endpoints, the use of central site monitoring to characterize exposures for the entire cohort, and by the traditional epidemiologic approaches used to examine exposure-effect associations.

In Project 1 we propose to build upon our initial analyses of the NAS cohort using novel exposure and epidemiological approaches designed to link particle components and their sources to biological pathways. This approach uses pharmacological and natural interventions as well as genetics, to highlight specific biological pathways. Specifically, we propose to collect ECG, blood inflammatory marker, medication, genotypic, food frequency, and particle exposure data for each of the 700 current NAS participants. ECG and blood marker samples will be analyzed for a variety of measures (HRV, ST segments, QT intervals, CRP, sICAM-1, sVCAM-1, and homocysteine) that will serve as intermediate markers of the inflammatory, endothelial, and autonomic pathways. These markers will be related to individual-specific PM<sub>2.5</sub>, SO<sub>4</sub><sup>2-</sup>, BC and trace element exposures that will be measured inside each participant's home for one-week prior to his/her clinic visit and to ambient air pollution (PM<sub>2.5</sub>, PM<sub>10</sub>, PM<sub>2.5-10</sub>, SO<sub>4</sub><sup>2-</sup>, NO<sub>3</sub><sup>-</sup>, BC, EC, OC, NO<sub>3</sub><sup>-</sup>, PC, and trace elements) concentrations that will be measured at our stationary ambient monitoring (SAM) site. The long-term (i.e., annual) effects of specific particle component exposures on measured markers of inflammation, endothelial function, and autonomic function will also be examined using a GIS-based exposure model.

The importance and relevance of the inflammatory, endothelial and autonomic pathways of particle toxicity will be examined using naturally occurring variations in the NAS cohort in: (a) the GSTM1 and HO-1 genotypes; (b) dietary micronutrient intake; (c) hypertensive and cardiac medication use, and; (d) methacholine reactivity. By examining how these variations modify exposure-effect relationships, natural interventions will be created that enhance or diminish the importance of the inflammatory, endothelial, and autonomic pathways. Given these variations, structural equation models, a powerful technique that combines multiple regression and factor analysis methods, will be used to examine relationships among the multiple PM components and health outcomes and to determine whether these relationships are consistent with specific biological pathways.

## 1. OBJECTIVES/HYPOTHESES

**Hypothesis 1:** Cardiovascular effects of particles (PM) will differ by source and by different source-related components. Specifically, short-term exposures to sulfate and traffic particles will be associated with increases in:

- **acute inflammation and/or endothelial dysfunction**, as measured by increases in CRP, soluble intercellular adhesion molecule 1 (sICAM-1), and soluble vascular cell adhesion molecule 1 (sVCAM-1);
- **autonomic dysfunction**, as measured by reduced heart rate variability (HRV) and;
- **general cardiovascular responses**, as measured by increases in blood pressure and ECG changes including ST-segment level and QT-interval.

**Hypothesis 2:** Effects of PM on these outcomes will be modified by subject characteristics (genetic, dietary, or pharmacological) that influence susceptibility to:

- **oxidative stress, endothelial dysfunction, and/or acute inflammation**, specifically Glutathione-s-transferase (GSTM1) null or the long repeat Hemeoxygenase-1 (HO-1) genotypes; statin, beta blocker, or calcium channel blocker use; dietary intake of Vitamin C or omega-3 ( $\Omega$ -3) fatty acids;
- **autonomic dysfunction**, specifically beta blocker use, calcium channel blocker use or dietary intake of  $\Omega$ -3 fatty acids;
- **general cardiovascular disease**, specifically hypertension and;
- **reactive airways disease**, specifically methacholine reactivity.

**Hypothesis 3:** Long-term exposure to PM from traffic is associated with increased risk of inflammation (e.g., CRP, sICAM-1, sVCAM-1, and homocysteine); autonomic dysfunction (e.g., reduced HRV), and impaired cardiovascular outcomes (e.g., elevated blood pressure). This association is modified by the same factors that modify acute responses.

## 2. INTRODUCTION

In 1997, the lack of plausible biological mechanisms was a major criticism of the new epidemiologic-based national fine particulate mass (PM<sub>2.5</sub>) standard. Since that time, substantial progress has been made, as studies have implicated numerous potential mechanisms by which particles may impact health. However, the relative importance of these potential pathways and the steps along these pathways are not well understood.

Identification of the relevant PM-mediated biological pathways has been hampered by several factors. Relevant biological pathways, for example, are likely to be PM component or source specific, as individual particle components, especially those from different sources, have been associated with different health responses<sup>1</sup> or have been shown to have varying toxicities<sup>2</sup>. These different mechanisms of action, however, have been difficult to disentangle, as ambient concentrations of PM components are often highly correlated with each other, making separation of their quantitative effects and identification of biological mechanisms less reliable by epidemiological approaches. Further complicating this identification is the fact that epidemiological studies are not designed to identify biological pathways, but are rather intended to examine associations between PM exposures and specific health outcomes, from which biological mechanisms can be inferred. While providing important information, these inferences

are not sufficient, as they provide little information about important intermediate steps along a causal pathway between exposure and health outcome.

As part of the research conducted for our current EPA PM Center, we began to use epidemiological approaches to identify mechanisms of PM toxicity. Specifically, we examined air pollution mediated responses of individuals participating in the Normative Aging Study (NAS), a large prospective cohort living in Eastern Massachusetts. As part of this effort, we collected ECGs and blood samples from each study participant and analyzed these samples for HRV and CRP, respectively. As discussed in more detail below, we found ambient PM<sub>2.5</sub> and ambient black carbon (BC) concentrations to be associated with decrements in HRV, with these decrements greatest for hypertensive individuals. Ambient BC concentrations were further found to be associated with increased CRP and fibrinogen levels. These results suggest that the PM-mediated autonomic changes may be brought about through pathways involving the autonomic nervous system and systemic inflammation. Definitive identification of PM-mediated biological mechanisms was limited, however, by the lack of other intermediate cardiac and inflammation endpoints, the use of central site monitoring to characterize exposures for the entire cohort, and by the traditional epidemiologic approaches used to examine exposure-effect associations.

In Project 1, we propose to continue our study of the NAS cohort using a different approach. This approach, which borrows from studies that rely on pharmacological interventions and from analytical approaches used in social science research, will take advantage of the wealth of detailed medical and behavioral data available for each NAS participant, by using these data to construct natural interventions that highlight specific biological pathways. Specifically, we propose to collect ECG, blood samples, medication, genetic, food frequency, and PM component exposure data for each of the 700 NAS participants. ECG and blood samples will be analyzed for several measures that will serve as intermediate markers of the inflammatory, endothelial, and autonomic pathways. These markers will be related to PM component exposures that will be measured for each participant, providing a powerful individual-specific database with which to examine the PM-mediated cardiac responses.

The importance of these pathways in understanding mechanisms of PM toxicity will be examined using naturally occurring variations in the NAS cohort in: (a) the GSTM1 and HO-1 genotypes; (b) dietary micronutrient intake, specifically of vitamin C and  $\Omega$ -3 fatty acids; (c) hypertensive and cardiac medication use, specifically of  $\beta$ -blockers, Ca channel blockers and statins, and; (d) methacholine reactivity (Table 1). By examining how these variations modify pollution-effect relationships, natural interventions will be created that enhance or diminish the importance of the inflammatory, endothelial, and autonomic pathways. Given these variations, structural equation models, a powerful combination of multiple regression and factor analysis methods, will be used to examine relationships among the multiple PM components and health outcomes, and to determine whether exposure to different PM sources affects a given stage of the pathway once we account for its effect on previous stages. Below we provide a brief discussion of the importance of each of the proposed intermediate health markers to the PM-mediated effects on inflammation, endothelial function, and autonomic function.

**Table 1: Modifiers and Pathways of the Effects of PM Components**

Modifiers	Pathway
Group 1: <i>Genotypes:</i> GSTM1 null, HO-1 long <i>Dietary Intake:</i> $\Omega$ -3 fatty acids, Vitamin C <i>Medications:</i> Statins, Ca-channel blockers, $\beta$ -blockers	Inflammation and Endothelial Function
Group 2: <i>Dietary Intake:</i> $\Omega$ -3 fatty acids <i>Medications:</i> $\beta$ -blockers, Ca channel blockers	Autonomic Nervous System
Group 3: Methacholine reactivity	General Pulmonary Health (may be affected by autonomic function)
Group 4: Hypertension	General Cardiovascular Health (may be related to inflammation and autonomic function)

**2.1. Autonomic Function, Cardiac Risk and PM:** Heart rate and HRV (the variation in normal RR intervals) are under the control of both the central and peripheral autonomic nervous system, through a finely tuned interaction between vagal and sympathetic activity<sup>3-7</sup>. Several studies have shown long-term changes in HRV to be associated with impaired cardiac health. Reduced HRV, for example, has been found to predict poorer outcome in post-infarction patients for both 24-hour and 2- to 15-minute recordings<sup>4,8,9</sup>. Furthermore, the Framingham Heart study, a 4-yr follow-up study of 736 subjects with a mean age of 72 years, found reduced HRV to be predictive of increased cardiac mortality independent of age, sex, cardiac history, blood pressure, medication use, diabetes, cigarette smoking, alcohol consumption, and arrhythmias<sup>10</sup>. Support for these findings was provided by a follow up study, which found reduced HRV to predict new events for a cohort of 2,501 individuals initially free of clinical cardiac disease<sup>11</sup>. While less is known about the impact of short term changes in HRV, results from several studies suggest that these short term changes may also be important for health<sup>12</sup>. In particular, increased LF/HF ratios have been reported immediately preceding the onset of ventricular tachycardias (VTs) compared to control periods in studies of patients with implantable defibrillators<sup>13,14</sup>.

During the past five years, numerous studies have reported that ambient PM concentrations are associated with changes in HRV. In our Summer 1997 study of 21 older adults in Boston, for example, we found ambient PM<sub>2.5</sub> concentrations to be associated with a reduction in HRV (r-MSSD)<sup>15</sup>. This finding was confirmed in our Summer 1999 follow-up study (under review), with the association strongest for BC, a marker for traffic particles. We found similar PM-related decreases in HRV in a younger occupational cohort in Boston<sup>16</sup>. Interestingly, the association in these workers persisted during non-work days,<sup>17</sup> indicating that healthy, younger subjects are also sensitive to PM at ambient levels.

Epidemiological findings linking particulate exposures to autonomic nervous system effects are supported by results from our recent animal studies using drugs to examine the effects of particles on specific biological pathways in rats. In these rat studies, both intratracheal instillation of urban air particles (UAP, SRM 1649, 750  $\mu$ g/Kg) and inhalation exposures to concentrated ambient particles (CAPs) led to significant increases in heart oxidants and edema. Neither cardiac oxidative stress nor edema occurred when atenolol was administered prior to exposure, suggesting that particle exposures promote oxidant-mediated cardiac dysfunction and that sympathetic activation after particle deposition in the lung is critical for cardiotoxicity (in review). Thus, there may be a complex relationship between the autonomic nervous system and

oxidant mechanisms that lead to inflammation. Such relationships need to be explored in human studies.

**2.2. Inflammation and Endothelial Function, Heart Disease, and PM:** CRP, sICAM-1, sVCAM-1, and homocysteine are markers of the interrelated processes of inflammation and endothelial function, which play important roles in heart disease<sup>18</sup> and atherosclerosis<sup>19,20</sup>. Accordingly, CRP, sICAM-1, sVCAM-1, and homocysteine have been shown to be independently and jointly associated with increased cardiac risk<sup>21-24</sup>. In a prospective study of 28,263 healthy, post-menopausal women, for example, increased CRP and sICAM-1 were associated with increased risk of cardiac events<sup>20</sup>. Correspondingly, elevated levels of sICAM-1 were associated with the development of accelerated atherosclerosis in a case-control study of 14,916 middle-aged men, while sVCAM-1 predicted hospital events in angina patients<sup>25</sup>. Homocysteine, which inhibits NO release<sup>26</sup>, has also been associated with coronary artery disease measured radiographically<sup>27</sup> and with flow mediated dilation<sup>28</sup>, suggesting that homocysteine levels may be a marker of impaired endothelial function.

Recent studies have shown particulate air pollution to increase levels of these and other markers of inflammatory and endothelial function. For example, we found that acute increases in ambient PM levels were associated with an elevation in fibrinogen in a large epidemiological study<sup>29</sup>, with this effect strongest in participants with chronic obstructive lung disease. Peters and co-workers<sup>30</sup> recently reported associations between daily air pollution concentrations and increased plasma viscosity during a period of elevated air pollutant concentrations, while other researchers reported increases in fibrinogen in controlled human<sup>31</sup> and animal studies<sup>32</sup> of urban particles. In addition, in a London study, black smoke and NO<sub>2</sub> showed stronger associations with plasma fibrinogen, an intermediate marker of cardiovascular disease, than PM<sub>10</sub><sup>33</sup>. Correspondingly, a recent controlled human exposure study found that exposure to diesel particles for one hour at 300 µg/m<sup>3</sup> resulted in increased levels of peripheral neutrophils, and increased levels of sVCAM-1 and sICAM-1<sup>34</sup>.

Consistent with these results, our current PM Center-funded animal studies found positive associations between CAPs exposures and reactive oxygen species, as measured by *in vivo* chemiluminescence, in both the lung and the heart<sup>35</sup>. Evidence from our recent follow-up study indicates that these particle effects may be mediated by the ability to mount a response to reactive oxygen species (ROS), as the ROS response was blunted by pre-administration with n-acetyl cysteine (NAC), a precursor to reduced glutathione, a primary antioxidant defense in cells and the lung lining fluid<sup>36</sup>. NAC was found to have a similar blunting effect on the activation of NF-KappaB and an inflammatory cascade by Provo, UT ambient particles<sup>37</sup>. These findings suggest that individual variation in antioxidant defenses, such as that provided by genetic polymorphisms and Ω-3 fatty acid and Vitamin C intake, will modify pulmonary, cardiovascular, and systemic response to PM.

**2.3. Glutathione-transferase (GSTs), HO-1, and PM/ROS:** GSTs are a family of enzymes involved in the metabolism of ROS and xenobiotic compounds. Genetic polymorphisms of the GSTs are common, and have been shown to modify the response to air pollutants<sup>38</sup>. The GSTM1 null genotype, present in approximately 40% of the general population<sup>39</sup>, has been associated with increased biomarkers of inflammation after ozone exposure<sup>40</sup> and an enhanced nasal allergic response to diesel exhaust particles<sup>41</sup>. In children exposed to environmental tobacco smoke (ETS), GSTM1 null was associated with an elevated odds of developing asthma<sup>42</sup>. GSTM1 null

further interacts with ETS to increase the risk of coronary disease<sup>43</sup> and levels of CRP, sICAM-1, and sVCAM-1<sup>44</sup>.

In addition to its physiological role in heme degradation, HO-1 may influence inflammation and a number of other cellular processes<sup>45</sup>. HO-1 has been shown to have anti-inflammatory effects, and as a result, HO-1 limits tissue damage in response to pro-inflammatory stimuli. The transcriptional upregulation of HO-1 responds to many agents, including reactive oxygen/nitrogen species. HO-1 has a (GT)<sub>n</sub> repeat in the promotor region. Smokers with longer repeats in this region have been shown to have greater incidence of coronary artery disease (CAD) and emphysema<sup>46,47</sup>. Their role in ROS-based inflammation and their observed modification of tobacco smoke and other particle effects on the heart suggest that genetic polymorphisms in HO-1 and GSTM-1 may contribute to variations in the cardiac response of individuals exposed to particles.

**2.4. Omega-3 Fatty Acids and Cardiovascular Disease:**  $\Omega$ -3 fatty acids can reduce inflammation by modifying the substrate for leukotriene production and can directly affect cell behavior, including autonomic function, by changing the behavior of ion gates in membranes. Intake of  $\Omega$ -3 fatty acids has been found to reduce adverse cardiac events. In the GISSI-Prevenzione trial, for example, patients receiving the  $\Omega$ -3 fatty acid supplementation had a 30% reduction in cardiovascular death and a 45% reduction in sudden cardiac death. The reduction in sudden cardiac deaths in the  $\Omega$ -3 group was significant after only 3 months of treatment. The survival benefit was not explained by a reduced rate of non-fatal myocardial infarctions, suggesting that  $\Omega$ -3 fatty acids reduce cardiovascular mortality mainly due to their anti-arrhythmic properties. Similarly, in a prospective cohort study of 22,071 males followed for 17 years, participants in the highest quartile of  $\Omega$ -3 fatty acid levels had an 81% relative risk reduction for sudden death as compared to participants in the lowest quartile. Correspondingly,  $\Omega$ -3 fatty acid intake has also been shown to affect intermediate markers of cardiac health, including reductions in CRP levels<sup>48</sup>, increased HRV, increased arterial compliance<sup>49</sup>, decreased VCAM-1 and decreased plasma viscosity<sup>50</sup>, all cardiovascular risk factors associated with PM exposure. To date, the only direct evidence that  $\Omega$ -3 supplementation reduces PM-mediated cardiac effects is provided by preliminary results from a recent study of PM<sub>2.5</sub> on HRV in Mexico City.

### 3. PRELIMINARY RESULTS

Below we present results from our preliminary studies in Boston of particle health effects. In addition, we present results from our PM exposure assessment and monitoring studies, which have informed our proposed follow-up analyses of the NAS cohort.

**3.1. Air pollution and HRV in the NAS:** In a study funded by our current EPA PM Center, approximately 750 NAS participants were examined between 2000 and 2003. In this study, we found an association between reduced HRV and both PM<sub>2.5</sub> and ozone, after controlling for age, diastolic blood pressure, fasting blood glucose level, cigarette smoking, angiotension converting enzyme (ACE) inhibitor use, room temperature, season, and outdoor temperature. Associations with BC were slightly weaker than those for PM<sub>2.5</sub>, while other pollutants showed little association with HRV. The pollution associations were strongest for participants with coronary heart disease (for PM<sub>2.5</sub>) and hypertension<sup>51</sup> (defined as either taking hypertension medication or having an SBP >140 or DBP > 90). The effects of PM<sub>2.5</sub> and ozone on LF were muted or blocked in those taking either calcium channel blockers or  $\beta$ -blockers, while the HF response was not

affected. Effect modification was not observed for participants on ACE inhibitors, which reduce blood pressure via different cardiac pathways. Observed effect modification by calcium channel and  $\beta$ -blockers, but not by ACE inhibitors, provide clues regarding particle toxicity mediated mechanisms. A paper describing these results is under review.

**3.2. Air pollution trajectories and HRV in the NAS:** As part of our current EPA PM Center, we identified sources of air parcels over Boston on different days using the wind field model output from the National Oceanic and Atmospheric Administration (NOAA)<sup>52</sup> and the Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) model<sup>53</sup>. The HYSPLIT model calculates ‘back trajectories’ showing the path of air parcels before they arrive in Boston during the 36 hours prior to the time of the NAS subjects’ exam at the Boston VA hospital. In collaboration with Dr. Barbara Stunder of NOAA, we used factor analysis methods to cluster these trajectories by source region<sup>54</sup> and analyzed their association with HRV controlling for covariates as above. Trajectories from the northwest, which were associated with high concentrations of copper and zinc on PM filters<sup>55</sup> but not particularly high mass concentrations, were most strongly associated with decreases in HRV as determined by HF and the LF/HF ratio. These associations suggest that air pollution from the northwest may be more toxic than air pollution from other regions and that particle toxicity varies with particle composition.

**3.3. Blood Markers of Inflammation/Endothelial Function in the NAS:** As part of our current EPA PM Center, we examined the effects of several pollutants (BC, total particle count (PC), and  $PM_{2.5}$ ) on inflammatory markers and blood parameters for 701 NAS subjects between 2000 and 2003. We evaluated pollution lagged averaging periods from the time of measurement to one month before the exam. CRP and fibrinogen were elevated in association with BC concentrations both 48 hours and one week before the measurement, while cholesterol levels increased with BC exposures one-month before the measurement. Associations between CRP and BC concentrations were higher among hypertensive individuals. Consistent with this finding, associations between fibrinogen and BC levels were observed only among the hypertensive subjects.

**3.4. PM, Inflammation, and Endothelial Dysfunction in Diabetics:** As part of our current EPA Particle Center, we conducted cross-sectional analyses of the relationship between air pollution and both inflammation and vascular reactivity in over 200 participants of Boston area diabetes clinical trials conducted between May 1998 and 2002. Analyses were performed using baseline pre-intervention values. Ambient PM concentrations averaged 24-hr prior to testing were associated with significantly reduced endothelium-dependent vascular reactivity in diabetics, with evidence for an additional cumulative effect related to previous day’s exposure. Both BC and  $SO_4^{2-}$  appeared to have effects on vascular reactivity. A slightly smaller effect on endothelial-independent responses to nitroglycerin was observed, suggesting that particles may influence both endothelial and smooth muscle function. This influence may be mediated through autonomic function and other mechanisms. Furthermore, PM levels were also positively associated with sICAM-1, sVCAM-1, and von Willibrand’s factor, suggesting both endothelial activation and inflammatory responses to particles. A paper describing these results is in review.

**3.5. Boston Ambient Concentrations:** In Boston, ambient  $PM_{2.5}$  is comprised primarily of  $SO_4^{2-}$ , EC, and OC, with relatively low levels of  $NO_3^-$  and trace elements (Table 2)<sup>56</sup>. Of these components,  $SO_4^{2-}$  comprises the largest fraction of ambient  $PM_{2.5}$  mass, contributing about 60% and 40% of the ambient  $PM_{2.5}$  during the summer and winter, respectively<sup>57,58</sup>. Ambient EC and OC levels do not vary significantly over the year, with average monthly concentrations of 1.4 ( $\pm$

0.5)  $\mu\text{g}/\text{m}^3$  for EC and 2.7 ( $\pm 1.5$ )  $\mu\text{g}/\text{m}^3$  for OC. Together, EC and OC account for approximately 25% of ambient  $\text{PM}_{2.5}$  during the summer and 40% during the winter, with the seasonal variation in its contribution the result of seasonal variation in  $\text{SO}_4^{2-}$  concentrations. Correspondingly, we found power plants (the major source of particulate  $\text{SO}_4^{2-}$ ) followed by motor vehicles (the primary source of EC and OC) to be the largest contributors to  $\text{PM}_{2.5}$  using source apportionment methods with our Six Cities and South Boston data.

**Table 2:** Distribution of the Daily Mean Air Pollutant Concentrations and Weather\*

Measure	Units	N	Mean	Percentiles					
				5%	25%	50%	75%	95%	IQR
$\text{PM}_{2.5}$	$\mu\text{g}/\text{m}^3$	2047	11.6	3.8	6.7	9.8	14.7	25.1	8.0
BC	$\mu\text{g}/\text{m}^3$	1555	1.10	0.31	0.59	0.95	1.43	2.58	0.84
$\text{SO}_4^{2-}$	$\mu\text{g}/\text{m}^3$	971	3.10	0.8	1.6	2.4	3.8	7.8	2.2
PC	$10^3/\text{m}^3$	806	30.7	11.8	20.2	29.3	40.6	53.0	20.4
$\text{NO}_2$	ppb	2557	23.1	13.0	18.1	22.4	27.3	35.2	9.2
CO	ppm	2558	0.8	0.3	0.5	0.8	1.0	1.4	0.5
$\text{SO}_2$	ppb	2558	5.8	1.7	3.2	4.7	7.2	13.7	4.1
$\text{O}_3$	ppb	2552	23.8	7.0	15.3	22.8	31.2	44.2	15.9
Ave. Temp.	$^\circ\text{C}$	2553	11.0	-3.3	3.7	10.9	18.7	24.8	15.0
Min. Temp.	$^\circ\text{C}$	2553	7.1	-7.2	0.6	7.2	14.4	20.6	13.8
RH	%	2549	69.0	42.9	56.7	69.0	81.5	94.3	24.8

\* Averaged across multiple sites from July 11, 1995 through July 11, 2002.

Although concentrations in Boston tend to be slightly lower than levels in other large metropolitan areas, our previous findings have shown that there is sufficient variability in PM, its components, and gaseous co-pollutants to examine acute particle health effects, as evidenced by results from the numerous epidemiological panel studies conducted to date in Boston.

**3.6. Spatial Heterogeneity in Component-Specific Particle Concentrations:** The ability of SAM site PM concentrations to reflect corresponding exposures depends in large part on the extent of spatial heterogeneity in PM levels, which has been shown to vary with particulate component. Sulfate, a major component of  $\text{PM}_{2.5}$  in Boston, is a stable particle species that varies little outdoors across wide geographic areas<sup>58</sup>.

Studies of traffic-related particles have used tracers to reflect total traffic-related particulate emissions, of which EC and filter blackness are the most common, particularly for diesel exhaust. Both markers have been shown to vary substantially within a city, with variations related to local traffic sources<sup>59-61</sup>. In a study conducted in Harlem, New York City, EC concentrations on four geographically distinct sidewalks varied 4-fold between sites, while mean  $\text{PM}_{2.5}$  (mass) concentrations were relatively uniform. The variation in EC was associated with bus and truck counts on adjacent streets and at one site with a bus depot<sup>61</sup>. Similar results have been reported in European studies, including the Small Area Variation in Air Quality and Health (SAVIAH) study in the Netherlands<sup>60</sup>. Spatial variation in traffic-related particle concentrations is supported by results from our Center-funded exposure studies of approximately 60 Boston area homes. As yet unpublished data from these Boston area homes show 24-hr EC concentrations to vary spatially, with concentrations outside the homes differing by as much as 350%. Furthermore, 24-hr EC concentrations at the homes were only moderately correlated with concentrations measured at our stationary ambient monitoring site located near downtown

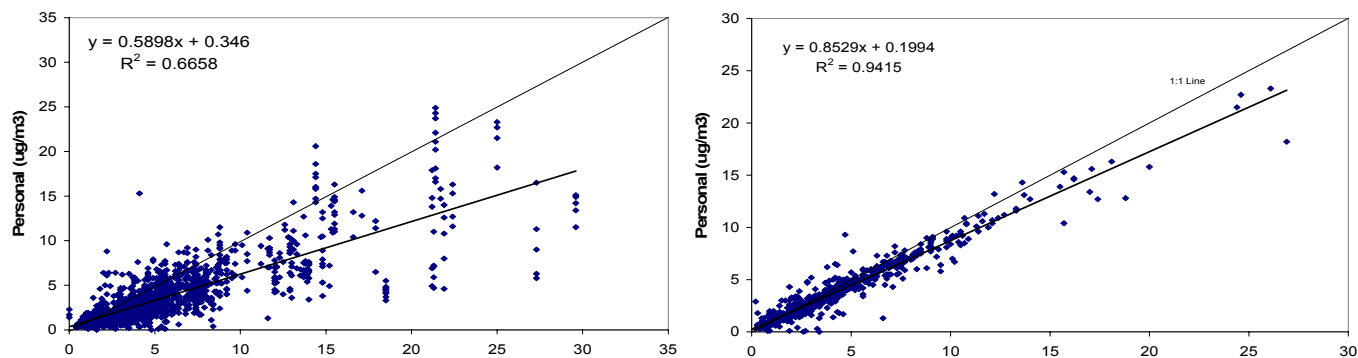
Boston. Home-specific correlation coefficients for comparisons between home and ambient EC concentrations from our study of 28 Boston homes had a median value of only 0.56 as compared to median correlation coefficients for  $\text{SO}_4^{2-}$  of 0.95. The spatial variability of EC concentrations, as reflected by the large ambient concentration differences, and the low median correlation coefficient is likely due to local vehicular emissions. These findings are consistent with previous exposure studies, which also suggest that the ability of a single central ambient site to reflect exposures for locally-generated particle components such as EC is weak<sup>62-66</sup>.

**3.7. Personal, Indoor and Outdoor Concentrations in Boston.** Over the past five years, numerous exposure studies have been conducted throughout the U.S. examining the associations between 24-hr personal, indoor and ambient  $\text{PM}_{2.5}$  (mass) concentrations. On average, 24-hr personal exposures have been found to be associated with corresponding ambient  $\text{PM}_{2.5}$  concentrations over time<sup>67-69</sup>. Associations between 24-hr personal exposures and ambient concentrations tend to be even stronger for  $\text{SO}_4^{2-}$ , a regional pollutant with few indoor sources, and weaker, but still significant for EC, a pollutant with abundant local sources<sup>61,70</sup>. These significant associations have made it possible for numerous epidemiological time-series studies to find associations between ambient  $\text{PM}_{2.5}$ ,  $\text{SO}_4^{2-}$  and EC concentrations and health effects using central site exposure data alone.

At the same time, however, the relationship between 24-hr ambient and personal exposures has been shown to exhibit considerable inter-personal variability, even for  $\text{PM}_{2.5}$  and  $\text{SO}_4^{2-}$ . This inter-personal variability is in large part due to variability in the fraction of ambient particles that penetrates indoors, which for  $\text{PM}_{2.5}$  has been shown to range between 0.3 and 1.0 depending on home ventilation rates<sup>69,71-74</sup>. As a result of this variable penetration, corresponding personal  $\text{PM}_{2.5}$ ,  $\text{SO}_4^{2-}$  and EC exposures for individuals living even within the same city may differ substantially for a given ambient concentration, raising concerns about exposure misclassification in health effect studies that rely on central site ambient concentrations alone as the exposure measure.

While personal and indoor  $\text{PM}_{2.5}$  measurements have been used to reduce exposure misclassification, they cannot be used as metrics of exposure to  $\text{PM}_{2.5}$  of ambient origin, since both personal and indoor  $\text{PM}_{2.5}$  exposures include both ambient and non-ambient sources. Instead, indoor  $\text{SO}_4^{2-}$  concentrations have been used as a more appropriate measure of exposures to  $\text{PM}_{2.5}$  from outdoor origin. Our studies have also shown that indoor home measurements are the best proxy measure of personal exposure. In our analysis of ambient, indoor, and personal exposure data from our panel study in Boston, MA, we compared average subject-specific personal/indoor and personal/ambient  $\text{SO}_4^{2-}$  ratios, and found that mean personal/indoor  $\text{SO}_4^{2-}$  ratios were equal to one in both the summer ( $1.00 \pm 0.11$ ) and winter ( $1.03 \pm 0.26$ ). In contrast, mean ratios of personal/ambient  $\text{SO}_4^{2-}$  concentrations were less than one in both seasons, with lower values in the winter ( $0.72 \pm 0.31$ ) as compared to summer ( $0.82 \pm 0.22$ ). Consistent with these findings, we found regressions of personal  $\text{SO}_4^{2-}$  exposures on corresponding indoor  $\text{SO}_4^{2-}$  concentrations in Boston, MA, Atlanta, GA and Steubenville, OH, to have slopes and  $R^2$  values were near one, while similar regressions with ambient  $\text{SO}_4^{2-}$  resulted in lower slopes and  $R^2$  values (Figure 1). Together, these findings suggest that indoor  $\text{SO}_4^{2-}$  measurements can provide a cost-effective and accurate means of assessing exposures to both  $\text{SO}_4^{2-}$  and PM of ambient origin.

**Figure 1:** Personal vs. Ambient (left) and Indoor (right)  $\text{SO}_4^{2-}$



Data from Boston, MA, Atlanta, GA, and Steubenville, OH. Each point represents one person-day. All data collected over 24-h periods, beginning at 8am.

#### 4. APPROACH

We propose to collect ECG, blood inflammatory marker, medication, genetic, food frequency, and PM component exposure data for each of the approximately 700 NAS participants. ECG and blood marker samples will be analyzed for a variety of measures that will serve as intermediate markers of specific biological pathways, including inflammation, endothelial function, and autonomic function. The importance of these pathways to particle toxicity will be assessed by examining whether the pollution-outcome relationship is modified by variations in: (a) the GSTM1 and HO-1 genotypes; (b) dietary micronutrient intake, specifically of vitamin C and  $\Omega$ -3 fatty acids; (c) hypertensive and cardiac medication use, specifically of  $\beta$ -blockers, Ca channel blockers and statins, and; (d) methacholine reactivity.

**4.1. NAS Population:** NAS is a longitudinal study of aging in Eastern Massachusetts established in 1963 by the Veterans Administration (VA). Community-dwelling men from the greater Boston metropolitan area were screened at entry and accepted into the study if they had no history of heart disease, hypertension, diabetes mellitus, cancer, peptic ulcer, gout, recurrent asthma, bronchitis, or sinusitis. Between 1963 and 1968, a total of 2,280 men were enrolled, ranging in age from 21 to 80 years (mean = 42 years) at entry. As of April 2004, 757 (mean =  $76 \pm 7$  years) of the 2,280 men continue to participate in the study. 17% of these men are diabetic, 24% have coronary heart disease, 22% have chronic obstructive pulmonary disease (COPD), and 58% are hypertensive. Correspondingly,  $\beta$ -blockers are used by 33% of the subjects, calcium channel blockers by 14%. 45% of the men are GSTM1 null.

**4.2. Routinely Collected Health Data:** As part of the original NAS study, physical examinations of each study participant occur every 3 years at the Boston VA Hospital. At each of these visits, extensive physical examination, laboratory, anthropometric, and questionnaire data are collected, including height and weight, a complete medical history, and sitting heart rate. In addition, sitting systolic (SBP) and diastolic blood pressures (DBP) are measured as the means of the left and right arm measurements. Blood samples are collected and analyzed for total serum cholesterol, high-density lipoprotein (HDL) cholesterol, fasting blood glucose (FBG) levels, white cell counts with differentials, and other standard parameters. Information about cigarette smoking, alcohol consumption, medical history (including respiratory and cardiac symptoms), and medication use are obtained by self-administered questionnaire. Each subject is interviewed to confirm the identity and purpose of medications used. Incidence of new disease is also noted. For all reported coronary diseases, hospital records are obtained and reviewed by a board

certified cardiologist<sup>75</sup>. Criteria used to confirm coronary diseases follow the established protocols used in the Framingham Heart Study and are classified using the 10<sup>th</sup> edition of the International Classification of Disease (ICD) codes 410-414. Subjects are recorded as having diabetes if they meet American Diabetes Association criteria (FBG levels greater than 126 mg/dL and/or physician-diagnosed diabetes)<sup>76</sup>.

**4.3. Additional Health Data Collection:** In addition to the regularly collected data, we propose to obtain additional data for each NAS participant at his/her clinic visit, including ECG measurements and daily diet information. In addition, we propose to analyze collected blood samples for additional parameters, including CRP, sICAM-1, sVCAM-1, and homocysteine.

**4.3.1. ECG Measurements:** Seven-minute long ECG measurements will be made for each participant while seated using a two-channel (five-lead) ECG monitor (Trillium 3000, Forest Medical, East Syracuse, NY). The ECG digital recordings will be processed, and heart rate and HRV measures will be calculated using PC-based software (Trillium 3000 PC Companion Software for MS Windows, Forest Medical). Only normal-to-normal (NN) beat intervals will be included in the analysis, with HRV measures calculated using the first four consecutive minutes of the recording without artifact. ECG recordings will be analyzed for HRV, ST segments, the length of the QRS complex, the QT interval, and for cardiovascular illness as determined using the Minnesota codes. HRV measures will include the standard deviation of NN intervals (SDNN), the square root of the mean of the squared differences between adjacent NN intervals (r-MSSD), total power (TP) (< 0.4 Hz), high frequency (HF) (0.15 to 0.4 Hz), low frequency (LF) (0.04 to 0.15 Hz), and LF/HF ratio. Subjects with atrial fibrillation, atrial bigeminy and trigeminy, pacemakers, irregular rhythm, irregular sinus rhythm, frequent ventricular ectopic activity, ventricular bigeminy, multifocal atrial tachycardia, or measurement time less than 3.5 minutes will not be included in the analysis.

**4.3.2. Food Frequency Questionnaire:** Subjects will be asked to complete a validated semi-quantitative food frequency questionnaire, which will be adapted from that used in the Nurses Health Study<sup>77</sup>. A total of 116 food items that account for most of the between-person variability in major nutrients will be listed, along with vitamin supplements. For each food item, a commonly served portion size or dosage will be specified. Participants will be asked to provide information on the average frequency with which food was consumed in the previous year. Nine possible choices will be listed for each food item, ranging from never to six or more times per day. Nutrient scores will be computed by multiplying the frequency of intake by the nutrient content of the food items. For vitamin intake, both total intake (including supplements) and dietary only intakes will be computed. Detailed data on fatty acid intake, including EPA, DHA, and total  $\Omega$ -3 intake, as well as  $\Omega$ -3 supplementation will also be determined.

**4.3.3. CRP, sICAM-1, and sVCAM-1:** Routinely collected blood samples will also be analyzed for CRP, sICAM-1 and sVCAM-1 in Dr. Ridker's laboratory at the Brigham and Women's Hospital, Boston, MA. CRP levels will be determined using a high sensitivity immunoturbidimetric assay on the Hitachi 917 analyzer (Roche Diagnostics, Indianapolis, IN) and reagents and calibrators from Denka Seiken (Nigata, Japan). sICAM-1 and sVCAM-1 will be measured in plasma in duplicate using the enzyme-linked immunosorbent assay method (R&D Systems, Minneapolis, MN) as has been used in previous studies<sup>78</sup>.

**4.4. Genotyping:** The potential for modification of the pollution-effect relationships will be examined for two genotypes GSTM1 and HO-long. For GSTM1, genotyping of the NAS

participants was performed at HSPH in 1995. The genotype assay involved PCR amplification of genomic DNA corresponding to exons 4 and 5 of the GSTM1 allele. Because these polymorphisms involve gene deletion, the assay was scored as positive for gene amplification or null, indicating homozygous deletion of the gene. Because the determination of the null genotype was based on the absence of PCR product, the CYP1A1 gene was used as a positive control and as such was co-amplified using the primers, resulting in a 312-bp product.

Genotyping for HO-1 will be performed in The Harvard-Partners Center for Genetics and Genomics using blood and/or DNA that has been archived for each of the NAS participants. Briefly, high-molecular-weight DNA will be extracted from the white blood cells with commercially available PureGene Kits (Gentra Systems, Minneapolis MN). Single nucleotide polymorphism (SNP) genotyping will be performed using MALDI-TOF (Matrix-assisted laser desorption ionization – time of flight) mass spectrometry as the main approach. All steps will be automated and will be tracked using a management system with bar-coding.

**4.5. Exposure Assessment:** PM exposures will be assessed using a three-tiered approach comprised of indoor, ambient and, GIS-based exposure data. This novel approach is designed to provide a cost-effective and accurate measure of each participant's PM exposures.

**4.5.1. Indoor Measurements:** At the core of our exposure assessment approach are one-week integrated PM<sub>2.5</sub>, SO<sub>4</sub><sup>2-</sup>, BC and trace element measurements that will be conducted inside each participant's home prior to his/her clinic visit. These indoor measurements will be used to provide an individual-specific metric of each participant's weekly pollutant exposure, as each measurement will take into account not only the inter- and intra-community variability in PM<sub>2.5</sub> levels, but also the variability in exposures introduced by home-specific differences, such as ventilation and activity patterns.

All indoor concentrations will be measured using our recently developed Microenvironmental Automated Particle Sampling System (MAPSS). This system, which is described in detail in the Technology and Monitoring Core, is lightweight, easy to use, quiet, and sturdy. The sampler will be shipped together with instructions via regular mail to each study participant prior to their scheduled health clinic visit. Boston VA Hospital staff will make all written and verbal correspondence. Upon receipt, the participant will connect the MAPSS to an electrical outlet and will place it on a table in the main activity room of the house (other than a kitchen). A battery-operated clock will allow a programmed timer to both start and stop the sample collection for the week-long period immediately preceding the participant's health clinic visit. Participants will be asked to bring the samplers with them to their health visit, with a telephone call made to each participant one day prior to their health exam as a reminder. We anticipate that a small number of participants will forget to return their sampler. For these participants, a self-addressed stamped mailer will be provided to them at their health visit, in which they will be asked to place the sampler in the mail. Upon their return, samplers will be sent to HSPH for laboratory analysis and reuse.

Indoor SO<sub>4</sub><sup>2-</sup> measurements will also be multiplied by the ratio of outdoor PM<sub>2.5</sub> (mass) to outdoor SO<sub>4</sub><sup>2-</sup> concentrations to estimate PM<sub>2.5</sub> exposures of ambient origin. Furthermore, indoor measurements will provide information about exposures to different particle source types, where PM<sub>2.5</sub> will serve as a measure of exposure to both indoor and outdoor particle pollution, SO<sub>4</sub><sup>2-</sup> as a measure of exposure to regional ambient pollution, and BC as a measure of exposure to local ambient pollution.

**4.5.2. Ambient Measurements:** The indoor measurements will be supplemented with continuous PM measurements from our SAM site located at the Countway Library, near the Harvard School of Public Health. Concentrations measured at this site will be used to characterize overall regional pollution for the entire study population. To account for spatial variation in locally generated pollutant concentrations and to provide more accurate daily measures of participant's exposures, 24-hr participant-specific, traffic-related particle concentrations will also be estimated using a GIS-based regression model. At our SAM site, continuous measurements of ambient PM<sub>2.5</sub>, PM<sub>10</sub>, PM<sub>2.5-10</sub> (by difference), SO<sub>4</sub><sup>2-</sup>, NO<sub>3</sub><sup>-</sup>, BC, EC, OC, and PC concentrations will be made, as will 24-hr integrated measurements of PM<sub>2.5</sub>, PM<sub>10</sub>, SO<sub>4</sub><sup>2-</sup>, NO<sub>3</sub><sup>-</sup>, and BC. All measurement methods are described in detail in our Technology and Monitoring Core.

**4.5.3. Measurements using GIS-models:** As described in the Biostatistics Core, 24-hr concentrations of traffic-related pollutants will be estimated outside each of our participant's homes using our recently developed GIS-based regression model for Eastern Massachusetts. This model is based on repeated observations from roughly 100 different locations, derived from State and Federal monitoring networks and from our previous exposure studies funded by our original Center and elsewhere. This model extends previous work by including both a regression approach (prediction by variables such as distance to road, etc.) as well as a geospatial smoothing term. In addition, by combining several surrogates of traffic particles (EC, BC, NO<sub>2</sub>), it allows us to obtain an estimate of traffic particle exposure using structural equation modeling that incorporates a measurement error correction. This model has been shown to predict 24-hr BC concentrations extremely well, resulting in an R<sup>2</sup> of 0.81 when 24-hr estimates were regressed on measured concentrations. Model performance is even higher when it is used to predict annual average BC levels. For this study, 24-hr outdoor home-specific concentrations of traffic-related pollutants will be estimated using ambient particulate concentrations measured at our SAM site and geocoded information for all of the current and past addresses of participants of the NAS. Annual averages will be computed by averaging the estimated 24-hr concentrations and will be used to test Hypothesis 3.

**4.6. Data analysis:** Established analytical approaches will be used to test our study hypotheses, as described in detail in our Biostatistical Core. Our principal outcomes will be continuous measures, with effect modification by numerous pathway specific parameters examined to test the hypothesis that PM component effects are modified by factors influencing susceptibility to oxidative stress, inflammation, and autonomic nervous system dysfunction (Table 1).

**4.6.1. Univariate Analyses:** Univariate analyses will be performed for all variables. Expected ranges for all of the variables will be defined a priori and out-of-range values or outlier values will be checked for errors. In addition to data cleaning, this initial analysis will serve the purpose of describing the study characteristics, identifying skewed variables that need transformation.

**4.6.2. Exposure Data:** As outlined above, exposures will be assessed for each individual using a three-step approach. In Step 1, hourly ambient pollutant concentrations obtained at the SAM site will be used to reflect exposures for the entire NAS cohort. In Step 2, individual-specific weekly exposures to ambient PM<sub>2.5</sub> will be determined by multiplying the weekly-integrated ambient PM<sub>2.5</sub> concentration by the estimated home-specific effective penetration efficiency, which is equal to the ratio of week-long indoor to outdoor SO<sub>4</sub><sup>2-</sup> concentrations measured in each home. Indoor SO<sub>4</sub><sup>2-</sup> concentrations will be obtained from indoor measurements made in each home, while week-long outdoor SO<sub>4</sub><sup>2-</sup> concentrations will be obtained using SAM site data.

Since homes will have integrated indoor concentration data for the week prior to the health measurements, effective penetration efficiencies will be assumed to be constant across this week. Similar methods will be used to estimate individual-specific weekly exposures to local ambient particles, with home-specific ratios of indoor to outdoor EC concentrations used to assess the effective penetration efficiency of these local particles. In Step 3, SAM site measurement data will be used in conjunction with GIS-based regression techniques to estimate yearly, traffic-related particulate concentrations outside each participant’s household. Finally, home-specific information on exposure factors, such as activity patterns and housing ventilation (including heating type, air conditioner type and use), and home age, type and size, will be incorporated into the health effects analysis and may provide indirect, but useful, information about types of exposures or activities that may have an effect on exposure-response relationships.

**4.6.3. Linear and Hierarchical Models:** Continuous outcomes will be handled as linear models, with hierarchical mixed models used to account for repeated measures and to examine effect modification. Effect modification by subject characteristics, such as hypertension status, genotype, medication use, or antioxidant intake, will be addressed with a random slope term, and will be examined at the second stage:  $v_i = a_i + a_1 * M$ , where  $M$  is a modifier variable as above.

**4.6.4. Covariate Selection:** Covariate selection will be outcome specific and will be based on biological factors and other study findings (Table 3). For all outcomes, variables will include age, sex, BMI, season, temperature, and day of the week. For HRV outcomes, covariates will also include fasting glucose and  $\beta$ -blockers and Ca channel blocker use. For the inflammatory and endothelial markers, variables will also include smoking, alcohol consumption, and statin use. Variables will be included in regression models regardless of statistical significance. If an association is found between our hypothesis variables and an outcome, sensitivity analyses will examine how that association changes as these other covariates are introduced into the model.

**Table 3:** Exposure, Covariates and Modifiers in the NAS

Exposure Variables	Covariates	Modifiers	Outcomes
PM <sub>2.5</sub> (hourly)	<i>Participant characteristics:</i> age, sex, BMI, fasting glucose, smoking	GSTM1 null	HRV
BC (hourly)		HO-1 Long	QT interval
PC (hourly)	<i>Meteorology:</i> temperature, season	$\beta$ -blockers	ST level
SO <sub>4</sub> <sup>2-</sup> (hourly)	<i>Time:</i> day of week	hypertension	blood pressure
O <sub>3</sub> , NO <sub>2</sub> , SO <sub>2</sub> , CO (hourly)	<i>Medications:</i> $\beta$ -blockers, Ca channel blockers, ACE inhibitors, statins	methacholine reactivity	CRP
24-hr and annual GIS based BC estimates	<i>Disease status:</i> diabetes, CHD, stroke	Vitamin C	sICAM-1
1-wk indoor elements, EC, SO <sub>4</sub> <sup>2-</sup> , PM <sub>2.5</sub>	<i>Diet:</i> alcohol consumption	$\Omega$ -3 fatty acids	sVCAM-1
Source Region (trajectories)		Ca channel Blocker	Homocysteine
		statins	

**4.6.5. Vulnerability Classifications:** Effect modifiers will be classified into three groups representing: (1) impaired ability to mount a defense against ROS, (2) pharmaceutical agents that reduce the ability of particles to stimulate a sympathetic response, and (3) pulmonary reflexes. ROS variables will be defined as the presence of any of GSTM1 null, HO-1 long, the low quintile of intake of Vitamin C, or the low quintile of intake of  $\Omega$ -3 fatty acids. Effect

modification will be tested for each variable alone and for a stepped modification with a three level variable that includes individuals that meet two or more of the ROS definitions. Reduced sympathetic response will be defined as any  $\beta$ -blocker use, any Ca-channel blocker use, or high quintile of  $\Omega$ -3 fatty acid intake. Again, a three category stepped variable will be defined with the high category representing  $\Omega$ -3 plus one of the medications. Pulmonary reflexes will be defined based on methacholine reactivity.

**4.6.6. Structural Equation Modeling (SEM):** SEMs are a class of covariance structure models that can be used to show path diagrams and to simultaneously model multiple surrogates of both exposure and outcome. The models are often represented as path diagrams, allowing one to fit models that specify outcomes as lying on a causal pathway between exposure and other outcomes. SEMs also allow for the specification of multiple surrogates of exposure on these pathways. See Section 4.3.1 in the Biostatistics Core (Core C) for a full description of SEMs, including important advantages of this modeling approach. We will fit several SEMs to NAS data. First, we will fit an SEM that considers the joint effects of short- and long-term exposure on ECG pattern via BP and an alternative pathway. In this model, we will specify short-term exposure as having a direct influence on BP. Results will allow us to distinguish between short- and long-term exposures, and potentially suggest that observed ECG changes can be attributed to effects on BP, with other pathways contributing little. We have successfully used this approach to separate out the effects of recent and longer-term lead exposure<sup>79</sup>. We will also fit a SEM that specifies an association between air pollution exposure and multiple blood markers thought to represent systemic inflammation. In particular, we will fit a model that specifies a single latent variable underlying the multiple outcomes CRP, sICAM-1, sVCAM-1, and homocysteine, and estimate the association between PM exposure and this latent variable. Because these outcomes are considered surrogates for systemic inflammation, the model allows us to avoid the multiple testing involved in fitting a model to each outcome separately, while gaining power by pooling information across multiple surrogates of the same underlying state. Finally, we will apply models that consider autonomic, inflammatory and endothelial pathways simultaneously. This model will allow us to investigate whether pollution is associated with one outcome (e.g., inflammation) via another (e.g., autonomic function). All of the SEMs will contain multiple surrogates of exposure to reduce the effects of exposure measurement error. For example, both BC and PC are dominated by mobile source emissions, and can be considered surrogates for a latent variable defined as traffic exposure. The latent biologic variables are subsequently modeled as a function of the common exposure, thereby adjusting out the measurement error associated with the individual exposure measures.

**4.6.7. Power Calculations:** As discussed in the Biostatistical core, power calculations for single outcomes use standard formulas for variance of regression coefficient estimates in a linear regression model. For repeated measurements on the same subject (as for HRV and CRP), we use variance formulas for linear regression and the fact that the mixed model can be expressed as a series of such regressions to assess the power to detect within-subject effects of pollution<sup>80</sup>. Because scientific interest focuses on both the main pollution effect and effect modification (as defined by GSTM1 gene, etc.), we calculated the power to detect both types of effects based on a cohort of size 600. For inputs, we used estimates of the between-person and (for the repeated measures cases) within-person variability for each outcome, as observed from existing studies conducted within our group. We used the existing estimates of pollution variability provided in the preliminary results of this project. For effect sizes, we assumed: (1) a 6.1 ms decrease in r-MSSD per 14.3  $\mu\text{g}/\text{m}^3$  increase in  $\text{PM}_{2.5}$ <sup>15</sup>; (2) a 147% increase in CRP per 100  $\mu\text{g}/\text{m}^3$  increase in

PM<sub>2.5</sub><sup>81</sup>; (3) a 48% increase in sICAM-1 per 14.3 ug/m<sup>3</sup> increase in PM<sub>2.5</sub>, obtained from our diabetic study, and: (4) a 2.72 mmHg increase in diastolic blood pressure per 20 ug/m<sup>3</sup>, as reported in Project 2 of this Center proposal. Table 3 shows that, under these assumptions, we will have at least 80% power to detect the main effect of pollution for all main effects of interest.

We also calculated the minimal detectable difference (MDD), with 80% power, for effect modification. We assumed that the less susceptible group exhibits the effect sizes listed above, and calculated the MDD in this effect for the more susceptible group for given subgroup sizes. Table 4 reports the MDD for a 20/80% split, which corresponds to effect modification by diabetes status. Power for effect modifiers with more even distributions, such as GSTM1 null and hypertension, is slightly higher. For instance, for a 60/40 split, the MDD for Diastolic BP is +2.7 versus +8.3 mmHg per 20 ug/m<sup>3</sup>, whereas the MDD for CRP is +147% versus +210% per 100 ug/m<sup>3</sup>. Analogous calculations for BC showed similarly high power for this pollutant. These power estimates are conservative in that they do not account for the spatial variability in BC as reflected in the proposed GIS model for exposure, as described in the Biostatistics Core.

**Table 4:** Power for Proposed NAS Study (n=600)

Outcome	Power Main Effect	Interaction MDD (20/80% cohort split)
HRV: r-MSSD	100%	-6.1 vs. -10.8 ms per 14.3 ug/mg <sup>3</sup>
Systemic Inflammation (CRP)	99%	+147% vs +305% per 100 ug/m <sup>3</sup>
Diastolic BP	80%	+2.7 versus +9.52 per 20 ug/m <sup>3</sup>
ICAM	100%	+48% versus 81% per 14.3 ug/m <sup>3</sup>

## 5. EXPECTED RESULTS

Results from this study will provide key information about the impact of ambient particles on inflammation, endothelial function, and autonomic function. By virtue of its size and detailed participant information, the NAS cohort will allow us to examine how participant-specific factors modify the impact of particle component exposures on cardiac health, which may help to identify effective interventions to protect against particle exposures. The use of SEMs will allow us to determine whether the causal pathways differ by particle source and will further our understanding of the relative importance of inflammation, endothelial function, and autonomic function to particle toxicity. As a follow-up to our original NAS study, the proposed study will provide an important link to our previous Center research. The proposed study complements other Center studies, as it is based on similar outcomes and pollutant measures.

## 6. GENERAL PROJECT INFORMATION

The study will be directed by Dr. Schwartz, the PI, together with the study co-investigators Drs. Suh, Vokonas, and Gold. The co-investigators will work with Dr. Bob Wright, who will conduct the remaining blood genotyping and genetic analyses. Drs. Schwartz, Suh, Gold, Speizer, Coull, Vokonas, and Koutrakis, together with other key study personnel, will meet bi-monthly to discuss study progress, study-related issues or problems, and coordination with other Center projects.

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