Project 2 (NAS):

Cognitive Decline, Cardiovascular Changes, and Biological Aging in Response to Air Pollution

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ABSTRACT – Project 2
a. EPA-RC2009-STAR-C1
b. Title: Cognitive Decline, Cardiovascular Changes, and Biological Aging in Response to Air Pollution
c. Investigators: PI Joel Schwartz, Co-PI: Murray Mittleman
d. Institution: Harvard School of Public Health, Boston, MA
e. Project Period and Location: 2010-2015, Boston MA
f. Project Cost: $1.5 Million
g. Project Summary

(1) Objectives and hypotheses: In this Project we will investigate the acute and chronic effects of air pollution on cognitive and neurological impairments, systemic inflammation, and vascular dysfunction. We will determine how these effects differ depending on the composition of multi-pollutant mixtures and the source contributions to PM composition. We will then ascertain the level of increased effects in susceptible and vulnerable subpopulations by examining modifying factors of obesity, diabetes, diet, socioeconomic position, and psychosocial stress.

(2) Experimental approach: Project 2 will build on our previous success using the Normative Aging Study (NAS) cohort, a large prospective cohort living in Eastern Massachusetts, and expand to make use of its extensive characterization for cognitive performance and psychosocial stress. With so much data already collected in this cohort, we can look at health effects longitudinally, where subjects act as their own controls. This reduces the potential for confounding while also increasing power. Our investigation of the health effects of air pollution will use our novel exposure approach to examine effects of individual pollutants, multi-pollutant mixtures, and sources. Under our current EPA Center grant, we developed and validated a spatio-temporal model for Black Carbon (BC) in the greater Boston area. Building on that success, we will now add a spatio-temporal model for O₃ to capture the spatial heterogeneity of exposure in the cohort study region, and we will estimate spatio-temporal variations in PM₂.₅, and longer term variations in composition of air pollution, as described in the Exposure core. Using these improved exposure models will allow us to extend previous findings to understand the impact of different components and combinations of air pollutants on different aspects of health. In combination with Project 1 and 5, we will examine these exposures across a spectrum from biomarker to mortality, and in combination with Projects 3 and 4, across the lifecourse. Finally, we will examine the differential effects due to factors of susceptibility and vulnerability.

(3) Expected Results: We have already reported different associations of traffic vs. secondary particles and ozone with different endpoints. With better exposure characterization and longer follow-up we will identify the key aspects of pollution that drive the association with cognition, inflammation, and vascular function. We will also determine the extent to which susceptibility factors, such as obesity, diabetes, and diet, modify these associations. This is critical for risk assessment, and will grow in importance as the prevalence of these conditions increases. Developing evidence suggests that stress and socioeconomic position may modify these health outcomes, and we will investigate the extent of these effects as well.

Supplemental Keywords: Air pollution, ambient particles, multi-pollutants, cognitive effects, vascular function, inflammation, neurological impairment, susceptibility, susceptibility and vulnerability
1. OBJECTIVES/HYPOTHESES

1.1 Specific Aims

We hypothesize that exposures to individual air pollutants, sources and pollutant mixtures are associated with cognitive and other neurological impairments, with vascular changes, and with changes in systemic and endothelial inflammation. We also hypothesize that different individual pollutants, sources and pollutant mixtures impact these outcomes differently. Moreover, we hypothesize that some of the heterogeneity in previous examinations of air pollution health effects have derived from variation in pollution composition and sources, from interactions among pollutants, and from variations in subject susceptibility and vulnerability. Our study aims to capture these variations, providing both greater power and greater understanding.

**Aim 1:** To identify the chronic effects on cognitive and neuropsychological function (both level and rate of decline), as indexed by repeated measurements of the Mini Mental State Exam (MMSE) and selected tests from CERAD, WAIS-R and NES2, of long-term exposure to individual pollutants, sources and pollutant mixtures.

**Aim 2:** To determine the acute and chronic effects on vascular and endothelial function, using repeated measurements of pulse wave analysis and blood pressure, of short-term and long-term exposures to individual pollutants, pollutant mixtures, and sources.

**Aim 3:** To determine the acute and chronic effects on inflammation, endothelial function, and oxidative stress, as reflected in repeated measurements of serum biomarkers, of short-term and long-term exposures to individual pollutants, pollutant mixtures, and sources.

**Aim 4:** (a) To investigate the modifying effect of relatively static measures of susceptibility (clinical/biologic measures) and vulnerability (social milieu), on the previously established relationships between individual pollutants, sources and pollutant mixtures and health outcomes; and (b) To explore the dose-response relationship between the potentially significant associations above and exposures to individual pollutants, mixtures, and sources, overall and by source and mixture, using flexible modeling techniques.

**Aim 5:** To examine the acute and chronic effects on Telomere length of short-term and long-term exposures to individual pollutants, sources, and pollutant mixtures.

1.2 Background and Introductory Information

1.2.1 Cognitive and Neurotoxic Chronic Effects of Air Pollution: Recent studies suggest there are chronic effects of air pollution on cognitive function. We found that higher exposure to traffic particles at home address of school-aged children living in Boston was associated with decrements in cognitive function (~2 IQ points). An association between traffic pollution and cognitive impairment in children was observed in another recent study. In adults, we reported associations between O₃ and reduced cognitive function, with confounding by socio-economic factors. Calderon-Garciduenas et al found that children living in Mexico City performed significantly poorer in cognitive tests (WISC-R) compared to children in a less-polluted city. They also found that MRI evaluation of the brains of children living in more polluted locations revealed greater prefrontal lesions, and highly exposed children and young adults showed upregulation of cyclooxygenase-2, IL1-β, and CD14. Dogs from Mexico City had greater rates of prefrontal lesions, neuro-inflammation, gliosis, and particle deposition. Particulate matter exposure has also been associated with increased inflammatory responses in the brain and altered neurotransmitter levels. Humans exposed to diesel exhaust had electro-encephalographic patterns indicative of cortical stress. More generally, particles and O₃ have been shown to increase oxidative stress, which is known to be relevant for neurodegenerative diseases.
Additional toxicological evidence suggests two mechanisms by which particles may affect the brain. Experimental protocols have shown that particles translocate retrogradely from the nose via the olfactory nerve into the brain.\textsuperscript{11} Such exposures extended not only to the olfactory bulb, but also to the striatum, frontal cortex, and cerebellum.\textsuperscript{12,13} Hence, one hypothesis is that the presence of particles in the brain due to the migration of particles from the nose to the brain causes the resultant effects. An alternative hypothesis is that ambient particle inhalation has a primary effect on the vasculature in the central nervous system (CNS). Project 1 will study the blood flow and resistance of the vasculature of the CNS directly, so our collaboration will give us additional insight into the mechanisms by which particles may affect the brain.

Our team has over a decade of experience studying cognition in the NAS, and recently demonstrated that cognitive decline was associated with lead exposure in that cohort,\textsuperscript{14} and was modified by psychosocial stress\textsuperscript{132}. Section 1.3 Progress Report describes our studies to date on air pollution in the cohort. Our new Center is designed to identify differential effects of pollutants, mixtures and sources on both level of cognitive function and rate of cognitive decline, and sort out interactions by both individual and community levels of psychosocial stress and socio-economic status, as well as other conditions that may modify responses.

1.2.2 Vascular and Endothelial Function: Blood pressure (BP) is a well-established intermediary biomarker predictive of cardiovascular events including stroke, myocardial infarction, and mortality. The role of air pollution in raising blood pressure is still unclear. We found that acute exposure to PM\textsubscript{2.5} was associated with an increase in BP in a study of Boston subjects with a resting heart rate ≥70 bpm.\textsuperscript{15} Other studies have also found associations between exposures to PM\textsubscript{2.5} or PM\textsubscript{10} and increased BP.\textsuperscript{16-18} However, these results have been inconsistent across studies,\textsuperscript{19,20} indicating a need for further research. Our collaborators in Project 1 showed that particle exposure increases blood pressure, and also increases baroreceptor sensitivity.\textsuperscript{20} This parallels our recent results in the NAS, which show that particles increase blood pressure\textsuperscript{21} and postural change in blood pressure.\textsuperscript{22} We have preliminary data showing long term exposure to traffic particles increases BP, and, similar to the experimental work of Bartoli et al, this result is modified by alpha-adrenergic blockage.\textsuperscript{23} While these studies suggest a key role for primary traffic particles, more detailed examination of pollutant sources and mixtures is needed, and differential PM toxicity depending on the source or the combination with other pollutants could be a contributing factor to inter-study variability in results. Our close collaboration with Project 1, and ability to replicate findings in Project 3, along with our ability to study PM in the context of multi-pollutant mixtures and by source contribution, give us the optimal position to elucidate the PM-induced effects on BP.

In elderly populations, central elastic arteries stiffen with advancing age, hypertension, or atherosclerosis, causing augmentation of central systolic and pulse pressures. The central and peripheral pressure waveforms can provide valuable information on arterial stiffness, as measured by augmentation pressure (AP) and Augmentation Index (Alx). Although limited, available evidence suggests that air pollution affects both AP and Alx. Exposure of healthy volunteers to diesel exhaust during moderate exercise caused a significant increase in AP and Alx,\textsuperscript{24} and similarly, healthy male nonsmokers exhibited a statistically significant increase in Alx both during and after exposure to ETS.\textsuperscript{25}

1.2.3 Serum Biomarkers: Urinary 8-hydroxy-2’-deoxyguanosine (8-OHdG) is an oxidative stress marker often used to assess the extent of repair of DNA damage (induced by inflammation and oxidative stress). Particles, and their associated metals, have been associated with increased 8-
OHdG production,\textsuperscript{26-30} with the strongest associations seen in exposure to traffic pollution.\textsuperscript{31-34} Bus drivers in Prague exhibited increased levels of 8-OHdG and other oxidative stress indicators vs. those in controls.\textsuperscript{35} Elevated 8-OHdG has also been reported in urban children compared to rural children.\textsuperscript{36} The inflammatory markers CRP, sICAM-1, sVCAM-1, and fibrinogen are independently and jointly associated with increased cardiac risk.\textsuperscript{37-41} For example, in a prospective study of 28,263 healthy, post-menopausal women, increased CRP and sICAM-1 were associated with increased risk of cardiac events.\textsuperscript{42} 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, and sVCAM-1 predicted hospital events in angina patients.\textsuperscript{43} Recent studies have found increased levels of inflammatory markers associated with particles. For example, we found that short-term increases in ambient PM levels were associated with an elevation in fibrinogen in a large epidemiological study, with this effect strongest in participants with chronic obstructive lung disease.\textsuperscript{44} Peters and co-workers have reported associations between air pollution and increased plasma viscosity.\textsuperscript{45} Other researchers reported increases in fibrinogen in controlled human studies of urban particles.\textsuperscript{46} Long-term (30-year avg.) exposures to both traffic-NO\textsubscript{2} and heating-SO\textsubscript{2} emissions in Stockholm were associated with increased serum IL-6 levels.\textsuperscript{47} In addition, in a London study, BC and NO\textsubscript{2} showed stronger associations with plasma fibrinogen, than did PM\textsubscript{10}.\textsuperscript{48} Also, a controlled human exposure study found that 1 hr exposure to diesel PM led to increased levels of peripheral neutrophils, sVCAM-1 and sICAM-1.\textsuperscript{49} Endothelial dysfunction can be induced by inflammatory mediators such as TNF-\textalpha, interleukin-1\textbeta (IL-1\textbeta), IL-8, and homocysteine which can enhance binding of low-density lipoprotein (LDL) to endothelium and upregulate expression of cell adhesion molecules. Homocysteine, which inhibits NO release,\textsuperscript{50} has been associated with coronary artery disease measured radiographically\textsuperscript{51} and with flow-mediated dilation.\textsuperscript{52} We found a positive association between traffic-related particles (BC and organic carbon) and total plasma homocysteine.\textsuperscript{53} We also found a positive association between acute PM\textsubscript{10} exposure and plasma homocysteine, with a stronger effect seen in smokers, suggesting that a preexisting proinflammatory condition increases risk.\textsuperscript{54}

1.2.4 Telomeres: Telomeres are regions of non-coding DNA at the ends of chromosomes that protect against structural degradation, inappropriate recombination, and end-to-end fusion of chromosomes.\textsuperscript{55,56} Telomere length declines with each successive cell division and thus serve as a measure of biological aging.\textsuperscript{57} Shorter telomeres are associated with greater risk of various chronic diseases, including diabetes,\textsuperscript{58} hypertension,\textsuperscript{58,61} atherosclerosis,\textsuperscript{62} coronary artery disease,\textsuperscript{63,64} heart failure\textsuperscript{65} and increased cardiovascular risk.\textsuperscript{66,67} Evidence from in vitro and human studies suggests that oxidative stress and inflammation accelerate telomere shortening. Reduced blood DNA telomere length has been also related to cumulative long-life exposure to tobacco smoking.\textsuperscript{71,72} Adverse effects of air pollution (oxidative stress, atherosclerosis, cognitive decline, increased blood pressure) to some extent mimic the effects of aging. Therefore, we will investigate whether environmental factors that influence oxidative and inflammatory responses also decrease telomere length, and whether decreased telomere length modifies the effects of pollutants.

1.2.5 Susceptibility (Obesity, Diabetes, N-3 Fatty Acids): The increasing prevalence of obesity and diabetes make these susceptibility factors especially important to study. There is evidence that obese and diabetic individuals are more susceptible to the effects of air pollution, putting a growing number of people at increased risk. In a 2002 study of 4 US cities we found that
diabetics have double the risk of a PM$_{10}$-associated cardiovascular admission compared with nondiabetics.\textsuperscript{73} Similarly, we estimated a 2.0-fold higher mortality risk associated with PM$_{10}$ exposure for diabetics than for controls in a 2004 case-crossover study.\textsuperscript{74} Likewise, PM$_{10}$ effects on mortality were stronger in diabetics than in non-diabetics in 9 Italian cities.\textsuperscript{75} Research also supports the hypothesis that obesity is a susceptibility factor for the acute cardiovascular effects of fine particles. Obese individuals were found to have twice the PM$_{2.5}$-induced reduction in standard deviation of all NN intervals (SDNN) than non-obese individuals, and had more PM$_{2.5}$-mediated HR increases.\textsuperscript{76} We have also examined diabetes and obesity as a susceptibility factors to air pollution in our current PM Center grant (Progress Report, Section 1.3). Extending these analyses to examine components, sources, and mixtures is a key goal of this grant. In addition to medical conditions, diet may influence the cardiovascular response to air pollution, with the strongest evidence for N-3 fatty acids. The GISSI study reported a protective effect of N-3s against arrhythmias,\textsuperscript{77} and a randomized trial found they reduced the effect of particles on heart rate variability.\textsuperscript{78} Additional research is needed to better understand the sources and severity of the increased risk to these subpopulations, and for additional endpoints.

1.2.6 Vulnerability (Psychosocial Stress): Psychological stress results when external demands exceed an individual’s perceived abilities and resources to meet those demands,\textsuperscript{79} and has been consistently linked to cardiovascular and other diseases.\textsuperscript{80} The Institute of Medicine reported that potential social causes of neurodevelopmental disabilities, including social isolation and psychosocial stress, have not been well studied.\textsuperscript{81} Recent animal studies and epidemiologic data suggest that social context modifies environmental neurotoxicants.\textsuperscript{82} In poor communities, social and chemical childhood stressors can jointly alter development and organization of the central nervous system.\textsuperscript{83} Animals in social isolation have increased neurotoxic effects from lead exposure,\textsuperscript{84} and furthermore, rats in an enriched social environment following lead exposure have shown reversal of learning impairment, increased gene expression of hippocampal NMDA receptors, and increased induction of brain derived neurotrophic factor mRNA.\textsuperscript{85,86} In the NAS, we have demonstrated stress-related modification in the relationship between bone lead and hypertension.\textsuperscript{87} Limited but growing epidemiological evidence suggests that psychological stress may also alter susceptibility to air pollution exposures. Social stress has been shown to modify traffic-related air pollution effects on asthma etiology\textsuperscript{88} or exacerbation.\textsuperscript{89} Some studies suggest psychosocial functioning may influence oxidative stress among adults, though the work is largely cross-sectional with small sample size. Among adults, 8-OHdG has been linked to depressive symptoms,\textsuperscript{90} clinical depression,\textsuperscript{91,92} perceived stress and perceived impossibility for alleviating stress,\textsuperscript{90} and caregiving for advanced cancer patients (a measure of chronic stress).\textsuperscript{93} Also, some air pollutants and psychosocial stress may independently affect common physiologic processes such as oxidative stress\textsuperscript{94} or inflammatory cell (IgE) production.\textsuperscript{95} Psychosocial stress has also been linked to telomere length. Shorter telomere length has been associated with higher levels of acute stress hormones,\textsuperscript{96,97} symptoms of depression,\textsuperscript{98} and high levels of emotion dysregulation.\textsuperscript{99} In addition, reduced telomerase activity has been associated with trait negative mood, perceived stress, chronicity of stress, as well as with behavioral and biological risk factors for heart disease.\textsuperscript{12,96} Given the evidence to date that both psychosocial stress and air pollutants may influence oxidative stress and cellular aging processes, understanding their interaction should both help explain variations in effect size, and the distribution of risk in the population.

1.3 Progress Report
The NAS cohort has informed us about toxicity of PM$_{2.5}$, BC, O$_3$ and SO$_4^{2-}$ on autonomic dysfunction, inflammation, and endothelial dysfunction. We have also gained new insights on
individuals at higher risk, such as those with obesity or diabetes. Moreover, we have studied genetic susceptibility factors that inform us about both at-risk groups and possible mechanisms of action of PM. We began exploring multiple pollutants and interactions, and new outcomes including cognition and biomarkers of oxidative stress, key to our proposed Aims. We highlight results from our NAS studies below.

1.3.1 Multiple pollutants and interactions: We have extended beyond our single-pollutant models to investigate multiple-pollutant models and pollutant interactions. We have demonstrated that higher cumulative lead exposures (predominantly from past gasoline lead) modified associations between air pollution and heart rate variability (HRV) among our NAS subjects. We found graded, significant reductions in both high-frequency and low-frequency powers of HRV in relation to O₃ and SO₄²⁻ across quartiles of tibia lead. We also found that polymorphisms of the hemochromatosis (HFE) gene protected against the effects of PM₂.₅ on HRV. Two polymorphisms in the HFE gene (C282Y and H63D) are associated with increased uptake of Fe and other transition metals into cells as compared to the wild type genotype, hence our results suggest a role of transition metals in this PM₃ effect. Concerning air pollution sources and mixtures, we have examined the differences in impact of PM₂.₅ on HRV by air mass origin using “backtrajectories”. We found that the effects of BC on all HRV measures were strongest on days with southwest trajectories, while the strongest associations of HRV with O₃ occurred on days when air parcels came from the west. PM₂.₅, BC, and SO₄²⁻ were associated with increased LF/HF ratio on days related to local, slow moving air masses. We have also identified separate effects of PM₂.₅ and PM₁₀-₂.₅ on daily deaths, and we have demonstrated that particles high in SO₄²⁻ or Ni have larger impacts on daily deaths.

1.3.2 Black Carbon Spatio-temporal model: We developed a GIS-based spatial smoothing model to predict 24-hr BC levels as part of our current Center (see Biostatistics Core). We used this model to predict address-specific exposures for NAS subjects and for subjects in other Boston-area cohorts. We generated address-specific exposures for more than 100,000 subjects within eastern Massachusetts using our BC model, and we examined the impact of BC on mortality. We also used this model to generate BC exposure predictions at locations of singleton births in Eastern Massachusetts, and we examined the association between BC and birth weight, also investigating the increased vulnerability from area-based socioeconomic measures (SEP). We further examined this birth weight association by adjusting for the measurement error induced by the modeled exposure predictions. In Suglia et al., we examined the effect of address-specific annual BC levels on cognition in children using predictions from this model. We also identified an inverse association between address-specific annual BC and telomere length in NAS subjects, using predicted BC from this model (under review).

1.3.3 Cognition: We have yet to study the effects of air pollution on cognition in the NAS cohort, but we have investigated cognitive effects of lead exposure in the NAS, finding an association of lead with cognitive decline. We have also studied the neurobehavioral effects of ambient air pollutants in other cohorts. In adult subjects, we found consistent associations between O₃ and reduced performance in the Neurobehavioral Evaluation System-2. In a study of children, we observed associations between BC and decreases in the vocabulary, matrices, and composite intelligence quotient scores of the Kaufman Brief Intelligence Test, and with decreases on the visual subscale and general index of the Wide Range Assessment of Memory and Learning.
1.3.4 **Inflammation, Endothelial Function, and Oxidative Stress:** We observed positive associations between traffic-related PM (PN and BC) and inflammatory markers (CRP, WBC count, sediment rate, and fibrinogen) among subjects in the NAS cohort. A follow-up study looking at repeated measures of CRP and Fibrinogen is now under review. We also found that exposures to traffic-related PM (BC and organic carbon) were associated with elevated plasma total homocysteine. A follow-up in press shows effect modification by genes related to oxidative stress. PM$_{2.5}$ and Black Carbon were associated with increased vascular cell adhesion in subjects, this association was modified by obesity, and by genes. We report associations between 8-OHdG and both PM$_{2.5}$ and SO$_4^{2-}$, but not BC in a cross-sectional study in review. Our continuing measurements of 8-OHdG in NAS subjects will improve our estimates of oxidative stress effects, part of Aim 3 of this proposal.

1.3.5 **Autonomic Dysfunction:** We reported an association between short-term PM$_{2.5}$ exposure and decreased HRV. We found that subjects with wild type endothelial related genes (APOE, LPL and VEGF) showed stronger effects of PM$_{2.5}$ on HRV compared to those with hetero- or homozygous genotypes. We found a negative association between PM$_{2.5}$ and HRV in subjects with lower dietary intakes of methyl nutrients (folate, vitamins B(6) and B(12), methionine), but not in subjects with higher intakes, and the association was also modified by methyl-related gene polymorphisms in MTHFR and cSHMT genes. In addition, we found that genes related to oxidative stress modify this association. We recently demonstrated an association between PM$_{2.5}$ exposure and postural change in BP. In a randomized trial of a wood stove intervention in Guatemala, we found decreased particle exposure was associated with decreased blood pressure. We also found an association between short-term exposure to BC and BP, and between long term BC exposure at address and BP in subsequent studies. Paralleling the work of Project 1, we found effect modification by alpha-adrenergic blockers.

1.3.6 **Telomere Length:** We recently reported that exposure to traffic was associated with shortened telomere length in blood leukocytes in a study comparing traffic officers to controls. Preliminary results among nonsmokers in the NAS show an association between shorter telomere length and address-specific annual BC (under review). Our continuing collection of telomere data will allow us to follow-up this association in a more powerful longitudinal analysis, and extend it to look at other components, mixtures, and interactions, Aim 5 of this proposal.

1.3.7 **Susceptibility:** In addition to looking at genetic and dietary susceptibility factors, we have investigated obesity and diabetes as susceptibility factors for increased risk to air pollution responsiveness. We found obesity to be a significant susceptibility factor for O$_3$ acute effects on lung function, with twice the estimated decrease in FEV$_1$ due to O$_3$ in obese subjects compared to non-obese subjects. In addition, we found that obesity worsened the PM$_{2.5}$ effects on the HF component of HRV, and found a greater effect of traffic-related PM on inflammatory markers in obese individuals. We also saw increased effects of both PM$_{2.5}$ and BC on sVCAM for obese subjects compared to non-obese. In addition, we found larger associations between PM$_{2.5}$ and inflammatory markers (CRP, IL-6) in individuals with diabetes, obesity, and hypertension. We found that PM$_{2.5}$, PN, BC and SO$_4^{2-}$ were each associated with decreased vascular reactivity among diabetic, but not for non-diabetic subjects. We found positive associations between PM$_{2.5}$, BC, and SO$_4^{2-}$ exposures and inflammatory markers (ICAM, VCAM) among subjects with type 2 diabetes. Similarly, in the NHANES III, we found that metabolic syndrome modified the PM$_{10}$ effect on inflammatory markers.
1.3.8 Vulnerability (Socio-economic position, SEP): We have advocated examining vulnerability factors, such as SEP, as modifiers of air pollution responses. We proposed hypotheses on how air pollution and socioeconomic factors may interact to influence health and suggested steps to advance knowledge in this field, fill information gaps, and apply research results to improve public health in collaboration with affected communities. We have reported that education level, sex, and age modify the effects of both air pollution and extreme temperatures on mortality. In addition, we have examined the role of SEP in incidence and survival after heart attack, and demonstrated use of mapping techniques to examine heat vulnerability.

1.3.9 Vulnerability (Psychosocial Stress): Psychosocial stress is often characterized by high levels of negative emotions, including anger, anxiety, or depression. We have studied psychosocial stress extensively in the NAS cohort, although not under our current Center. For example, we found a positive interaction between psychosocial stress and lead on systolic BP, and found that highly stressed individuals had an increased risk of developing hypertension for a given increase in bone lead levels. We also found that psychosocial stress modifies the lead effect on cognition (in press). Furthermore, prospective studies within the NAS have consistently found psychosocial stress to be associated with incident CHD, where men with >2 anxiety symptoms showed elevated risks of fatal CHD and sudden death, and men with the highest levels of worry about social conditions had higher risks for nonfatal myocardial infarction and for total CHD. Additional work in the NAS found an increased risk of CHD associated with depression, as measured by the Minnesota Multiphasic Personality Inventory (MMPI), with effects persisting after adjustment for known cardiovascular risk factors. Another NAS study showed that baseline levels of general stress, anxiety, and depression were each strong predictors of incident CHD over the follow-up period, and anxiety and depression remained independent predictors when modeled jointly. We have work underway looking at the effect of stress on inflammatory markers, and on DNA methylation in this cohort.

1.3.10 Progress Summary: With the NAS cohort, we have demonstrated that short-term air pollution exposure is associated with acute changes in BP, CVD-related autonomic function, and intermediate biomarkers of inflammation and oxidative stress. We showed that diabetes, obesity, and relevant genetic polymorphisms modify these effects, and we have demonstrated expertise in studying the modifying effects of vulnerability factors of psychosocial stress and socio-economic position. Our continuing collection of data on augmentation index, telomere length, 8-OHdG in this large cohort will give us the power to examine effect modification by multiple sources of susceptibility and vulnerability. We have demonstrated our experience in analyzing cognitive functioning in the NAS in relation to other environmental exposures. We have used our spatio-temporal model of BC to identify associations between long-term exposure to BC and decreased telomere length, and we have studied PM$_{2.5}$ sources using back-trajectories. Our exposure modeling will expand the scope of our studies to include long-term exposures to pollutant mixtures and sources.

2. APPROACH

We propose to examine the effect of exposure to individual air pollutants, sources, and pollutant mixtures on measures of cognitive function, vascular function, autonomic function, telomere length, and intermediate biomarkers of inflammation, oxidative stress and endothelial function using the NAS cohort. Participant data has been analyzed for a variety of these measures, and we continue to collect data.
2.1 NAS Population: NAS is a longitudinal study of aging in Eastern Massachusetts established in 1963 by the Veterans Administration (VA). Starting in 1963, healthy, 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. The original study population included 2,280 men, ranging 21-80 yr in age (mean=42 yr). We have studied the NAS cohort during our previous two EPA Centers, working in close collaboration with researchers at the VA. The study population from 1995 onward includes >800 men (Table 1).

<table>
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<tr>
<th>Variable</th>
<th>Mean (SD)</th>
<th>Total N obs</th>
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<tbody>
<tr>
<td>Age, years (SD)</td>
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<tr>
<td>Body mass index, Kg/m² (SD)</td>
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<td>N=3,265</td>
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<td>Smoking status, n (%)</td>
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<td>Former smoker</td>
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<td>N=3,265</td>
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<td>Current smoker</td>
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<td>Pack years of smoking,* pack x year (SD)</td>
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<td>Diabetes, n (%)</td>
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<td>Stroke, n (%)</td>
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<td>VEGF, pg/mL (SD)</td>
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<td>Urinary 8-OHdG, ng/mL (SD)</td>
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<td>N=413</td>
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*Mean and SD among ever smokers

2.2 Health Data
As part of the original NAS study, physical examinations of each study participant occur every 3-5 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. 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. The cost of these visits and assays is borne by the VA.

2.2.1 Cognitive Tests: The NAS study includes a battery of cognitive tests. To assess global cognitive function, we administer the Mini Mental State Examination (MMSE), measured on 1,021 subjects from year 1995 onward. Since its creation, the MMSE has been validated and extensively used in both clinical practice and epidemiologic studies. The test assesses orientation to time and place, immediate and short-term recall, attention and calculation, word finding, construction (figure copying), reading and writing skills, and ability to follow a 3-step command. When administered repeatedly, the MMSE is able to measure changes in cognitive status. The other cognitive tests (administered since the mid 1990s) assess a broad range of neuropsychological functions, including verbal fluency, working memory, verbal learning and memory, constructional praxis, and visual analysis and discrimination, selected from the Wechsler Adult Intelligence Scale-Revised (WAIS-R), the Consortium to Establish a Registry for Alzheimer’s disease (CERAD) battery, and the NES2 (Neurobehavioral Evaluation System). The verbal fluency test from CERAD assesses the speed and ease of verbal production. The subject is required to generate as many words as possible within 60 sec from a specified semantic category. Poor performance on this task can result from difficulties with regard to vocabulary size, word retrieval, speed of response, mental organization, search strategies, and short and long term memory. This test is sensitive to cognitive impairment from a variety of etiologies including dementia. The digit span backward test from WAIS-R is a test of verbal memory, focusing on the span of immediate retention, in which the subject is asked to recall a list of random numbers in reverse order. This test assesses working memory, and executive function, although impairments of attention and comprehension can also contribute to poor performance. The word list memory test from CERAD is a test of immediate memory span, consistency of recall, and rate of learning new material, in which the subject must recall of list of words presented on the computer screen for three consecutive trials. This test can also be informative as an assessment of working memory. The constructional praxis test from CERAD is a drawing task that assesses the ability to integrate visual-perceptual skills with a motor response. Such tasks are known to be very sensitive to cognitive impairment. Participants copy a circle, crossed rectangles, vertical diamond, and a cube. The pattern comparison test from NES2 is a test of visual analysis and discrimination in which subjects choose the one different pattern among three patterns and are scored by the number of correct responses and the mean response latency for correct decisions.

2.2.2 Measures of Vascular and Endothelial Function: Pulse wave measurements are made in the right arm using the Sphygmocore Px (Atcor Medical Blood Pressure Analysis System, Australia) in a temperature-controlled room. After recording 20 sequential waveforms, the system software generates average peripheral and central waveforms. The software also computes the Augmentation Index, defined as the difference between the second and first peaks of the central arterial waveform, expressed as a percentage of pulse pressure. Sitting systolic (SBP) and diastolic blood pressures (DBP) are measured as the means of the left and right arm measurements. Postural BP (supine and standing) are also obtained.

2.2.3 Biomarkers of Oxidative Stress, Inflammation, and Endothelial Function: Routinely collected blood samples are analyzed for CRP, sICAM-1 and sVCAM-1 in Dr. Ridker's laboratory at the Brigham and Women's Hospital, Boston, MA. CRP levels are 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). Fibrinogen is assayed using the MDA Fibriquick method.\textsuperscript{151} sICAM-1 and sVCAM-1 are measured in plasma in duplicate using the enzyme-linked immunosorbent assay method (R&D Systems, Minneapolis, MN) as has been used in previous studies.\textsuperscript{152} Urinary 8-OHdG analysis is performed by Genox Corp (Baltimore, MD) using a competitive enzyme-linked immunosorbent assay.\textsuperscript{153,154} Total plasma homocysteine (tHcy) is measured from fasting plasma samples and assessed at the USDA Human Nutrition Research Center on Aging at Tufts University using high-performance liquid chromatography with fluorescence detection.\textsuperscript{53,155} Measurements of IL-1\textbeta; IL-6; IL-8; TNF-\alpha; VEGF, and sTNF-RII from serum samples (Milliplex Map, Millipore Corporation, MO) using Luminex xMAP multiplexing technology (Luminex® 100/200™ System Luminex, Austin, TX) housed and operated in the HSPH Molecular Epidemiology Laboratory. To determine telomere length, DNA is extracted from stored frozen buffy coat using the QiAmp DNA blood kits (QIAGEN, Germantown, MD, USA) and used for leukocyte telomere length (LTL) measurement by means of quantitative real-time PCR.\textsuperscript{156} All samples are run in triplicates, and the average T/S ratio is calculated by dividing the averages of the three T measurements by the three S measurements.\textsuperscript{157}

2.3 Markers of Susceptibility and Vulnerability

2.3.1 Susceptibility: Susceptibility factors include obesity, diabetes, and genetic markers. 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).\textsuperscript{158} Subjects with BMI $\geq$30 are classified as obese. Subjects are classified into tertiles of long term intake of N-3 fatty acids based on quantification of the Willet Semiquantitative Food Frequency Questionnaire.

2.3.2 Vulnerability: We administer the Health and Social Behavior Questionnaire to assess participant stress perception.\textsuperscript{159-161} Participants are asked to think of the most stressful thing that occurred to them in the past month and rate the stress level on a 7-point scale compared with other problems in the past, from “not troubled” to “the most troubled I’ve ever been.” The 14-item Perceived Stress Scale (PSS), a measure of the degree to which respondents felt their lives were unpredictable, uncontrollable, and overwhelming in the preceding 1 month, is also ascertained.\textsuperscript{162} Each item is scored on a 5-point scale ranging from “never” to “very often” and a composite score is obtained. The PSS is the most widely used stress appraisal measure; it correlates with life events scores and depressive and physical symptomatology, and has been shown to be a better predictor of a number of health outcomes compared with life-event measures,\textsuperscript{162} and it possesses substantial reliability and validity.\textsuperscript{163} An anxiety symptom scale was constructed of five items from the Cornell Medical Index and administered to the cohort at baseline: “Do strange people or places make you afraid?”; “Are you considered a nervous person?”; “Are you constantly keyed up and jittery?”; “Do you often become suddenly scared for no good reason?”; and “Do you often break out in a cold sweat?” These items were selected in an \textit{a priori} manner on the basis of their close resemblance to items included in existing, validated psychological assessment scales for anxiety, including the Brief Symptom Inventory and the Spielberger State-Trait Anxiety Inventory.

2.3.3 Relationship among modifiers: Standard risk assessment paradigms reduce the multidimensional aspects of risk (the risk of each person in the population, given their particular attributes) into a single estimate: the overall risk in the population, or equivalently, the mean risk. This averaging of risk assumes that the mean individual risk is small and it varies about this small average, but not enough to raise the risks in a definable subpopulation to a level of
concern. However, this is likely untrue. For example, factors of psychosocial stress and higher pollutant exposures tend to cluster in the poorest populations, contributing to further increases in risk to the effects of air pollution. Thus, the distribution of risk in the population could be highly skewed and the risk in identifiable subgroups due to differential susceptibility (including interactions with other risk factors) may be large. Additionally, the skewed distribution of risk can also involve more complex social factors or more than one interaction. For example, persons with diabetes have twice the risk of cardiovascular mortality following exposure to particulate air pollution as persons without the syndrome, and genes related to oxidative stress defenses modify the risk of air pollution. These risk modifiers are rarely independently distributed, nor do they occur randomly throughout the population. A risk assessment that seeks to capture the distributional aspects of risk must include the covariance of the risk modifiers, which can greatly increase the actual skewness of risk in the population. To do so, research must identify that covariance, as well as the interactions, and we propose to do this in our study.

2.4 Exposure Assessment

As highlighted in the Exposure Core, we have developed an exposure strategy that addresses scientific questions on the health effects of short-term and long-term exposures to individual pollutants, multi-pollutant mixtures, and sources. These exposure metrics will be common to all three cohorts, bringing greater strength to the interpretation and validation of the associated health effects. In the NAS cohort, we will examine both acute and chronic health effects of these exposures on the health outcomes identified in the Specific Aims: cognitive function, vascular and endothelial function, and serum biomarkers of inflammation and oxidative stress.

2.4.1 Short-term Exposures and Acute Effects: We will use short-term exposures to examine acute effects on vascular function, and on serum biomarkers of inflammation, oxidative stress, and endothelial function. Short-term exposures will be averages of one day, several days, or several weeks before each participant’s study visit, with the length of the time window depending on the specific health outcome examined. Short-term exposure metrics will include:

(a) Individual pollutants: Daily concentrations of O₃, PM₂.₅ mass, number, elements, ions, BC, EC and OC, VOCs, PM₁₀ and PM₁₀-₂.₅ will be acquired from the Boston HSPH Supersite and Boston city sites. We will estimate address-specific 24-hr exposures to BC and O₃ using GIS-based spatio-temporal modeling, and we will generate spatially (4km) and temporally resolved estimates of 24-hr PM₂.₅ exposure by calibrating satellite data with the spatial sites.

(b) Sources: We will estimate the daily contributions to PM source apportionment, including traffic, soil, coal combustion, and sea salt among others, from 1995 onward. These contributions will be estimated using the PMF EPA source apportionment method.

(c) Multi-pollutant mixtures: We will group days according to their multi-pollutant mixture profiles based on the daily composition all measured individual pollutant levels, using clustering methods. For each outcome, we will estimate the effect of PM₂.₅ using mixture as an effect modifier. This will make it possible to examine the relative toxicity of each mixture identified. Subsequently, we will link mixture toxicity to mixture characteristics and origin.

2.4.2 Long-term Exposures and Chronic Effects: We will use long-term exposures to examine chronic effects on cognitive function, on vascular function and on serum biomarkers of inflammation, oxidative stress, and endothelial function. Long-term exposures will be averages of one year or several years before each participant’s study visit, with the length of time depending on the health outcome examined. Long-term exposure metrics will include:
(a) Individual pollutants: For each participant, we will generate annual exposure averages to BC and O₃ (using our 24-hr spatio-temporal GIS models), and to PM₂.₅ (using our spatio-temporal estimates from re-calibrating the satellite data with the spatial sites). Additionally, we will use the new 2010-2014 monitoring data to build pollutant-specific seasonal spatial surfaces, and we will get spatially-resolved seasonal estimates for 1995-2009 by calibrating the surfaces with the 1995-2009 central site measurements and validating those estimates with the spatial monitor sites currently in place (approximately 20, see Exposure Core). From these seasonal-spatial estimates, we will compute annual averages for each participant, for all measured pollutants.

(b) Sources: Starting with the year 2000, we will run source apportionment models for all sites and all years, and then calculate season-specific averages of each source contribution for each site. Using spatial smoothing of seasonal source contributions, we will estimate geocoded annual mean source contributions.

(c) Multi-pollutant mixtures: Using the pollutant-specific seasonal spatial surfaces in (a), we will calculate annual averages of each pollutant species for each zip-code. Using clustering methods, we will group the zip-codes by annual pollutant mixture average. The mixture type for each zipcode will be examined as an effect modifier for the health effects of individual-specific annual PM₂.₅ exposure.

One-year average exposure: One-year average exposures to air pollution have been shown to be relevant exposure metrics in mortality studies. A follow-up analysis of the Six City Study looked at year-to-year changes in particle concentrations to examine the lag between change in exposure and change in mortality rate.¹⁶⁴ We showed that the association was essentially linear down to 8 µg/m³, where lower concentrations were too sparse in the data to identify linearity, and that the effects of reduced particle exposure on mortality appear to be mostly seen within two years. This conclusion is also supported by natural experiments. In the Utah valley when a strike closed a steel mill, mortality fell that year but returned to its previous level the next year, when mill operations resumed.¹⁶⁵ In another cohort study, we examined over 66,000 nurses living in the Northeast and upper Midwest.¹⁶⁶ Using a spatial model that estimated monthly PM₂.₅ concentrations at the addresses of each nurse, we found that a 10 µg/m³ increase in PM₂.₅ at a nurses address was associated with a 26% increase in risk of dying in that year. This increase was predominantly seen within a year of the change of exposure.

Full details of these exposure metrics are given in the Exposure and Biostatistics Cores.
2.5 Data analysis
Established analytical approaches will be used to test our study hypotheses, as described in detail in our Biostatistics Core. Our principal outcomes will be continuous measures, with effect modification by numerous pathway specific parameters examined to test the hypothesis that air pollution effects are modified by factors influencing susceptibility to oxidative stress, inflammation, and autonomic nervous system dysfunction.

2.5.1 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.

2.5.2 Covariate Selection: All models will include age, BMI, and temperature, and day of the week. Other known confounders will be included a priori, based on the specific outcome of interest. For example, a model for BP would include hypertensive medication use, incidence of CHD, and diabetes.

2.5.3 Linear and Hierarchical Models: The majority of our outcomes are continuous measures. We will use linear models for outcomes with only one measurement per person (8-OhdG, pulse wave measures). Hierarchical mixed models will be used for outcomes with repeated measures on each subject (MMSE, CERAD, WAIS-R, NES2, Telomere length, BP, ICAM, VCAM, CRP, IL6, IL1b, IL8, TNFa, VEGF, fibrinogen). Mixed models allow us to account for correlation among measurements on the same subject. Mixed models will have the form:

\[ Y_{ij} = (\beta_0 + u_i) + \beta_{age} + \beta_{bmi} + \beta_{dow} + \beta_{temp} + \cdots + (\beta_p + v_i) \text{pollution}_{ij} + \epsilon_{ij} \]

Specifically, \( Y_{ij} \) is the health response in subject \( i \) on day \( j \), and age, BMI, and temperature are covariates. Here \( u_i \) represents a subject specific intercept, reflecting unexplained heterogeneity in subjects’ overall level of outcome, and \( v_i \) the subject specific slope, representing heterogeneity in response. To examine effect modification by subject characteristics (such as having diabetes, being obese) we will use an interaction term to fit separate pollution slopes for each subgroup. Thus, we extend the model above by adding the interaction term: \( b_{p,subgroup} \text{pollution}_{ij} \cdot \text{Modifier} \).

For example, to estimate the acute effect of short-term exposure to PM\(_{2.5}\) on BP, we would fit the overall model with confounders mentioned above, and with address-specific 2-day PM\(_{2.5}\) exposure as \( \text{pollution}_{ij} \), and obtain the estimated \( \beta_p \). Then to identify which multi-pollutant mixture was linked with the strongest response, we would add indicator variables for each mixture type, and, assuming \( X \) types, use interaction terms with PM\(_{2.5}\) to obtain the estimates of \( \beta_{p,\text{mixture}_1}, \ldots, \beta_{p,\text{mixture}_X} \) and compare those estimates to see which mixture had the strongest effect.

2.5.4 Causal Modeling: Observational studies differ from experimental trials in that the exposure is not randomly assigned, and hence effects may be confounded. Causal modeling examines methods of analysis that offer the possibility of randomizing the exposure. All of the methods require an untestable assumption that the appropriate variables are used, but external knowledge can make those assumptions reasonable in some cases. One critical case involves time varying exposures such as air pollution. While air pollution shows some serial correlation, its correlation with past or future values falls off quickly with the time interval between them. In that case, a variant of G-estimation and Intensity Score estimation becomes feasible. Briefly, suppose we have repeated measurements of exposure 4 years apart. Instead of regressing outcome against exposure \( (X) \) at time \( t \), we can regress it against \( X_t - E(X|Z_t) \), where \( Z_t \) includes all previous values

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of $X$, of covariates, and of outcome. This is essentially the difference of exposure from what might have been expected given past history of everything. If we further assume this is uncorrelated with future exposures, which seems reasonable, it is possible, with some modifications described in Brumback et al.\textsuperscript{16} to treat this as a random exposure variable, and derive causal inferences from its coefficients, under the usual other assumptions.

2.5.5 Power: We have tested power in mixed models using a simulation approach, where 1000 samples are generated with plausible correlations across repeated measures of outcome (0.6) and exposure (0.3) after 3-5 years. For 750 subjects and 1, 2 or 3 repeated measurements per subject, we have at least 98% power to detect whether a 1 SD change in exposure produces a 4% change in outcome, for outcomes whose variance ranges up to twice that of systolic blood pressure. For interactions, with the same correlations, and a prevalence of 0.2 for the modifier, we have 68% power to detect modification. For modifiers with a prevalence of 0.3 in the population, power increases to 79% for the interaction.

2.5.6 Exposure-Response Relationship: We have demonstrated expertise in modeling the exposure-response relationship between air pollution and health effects to determine linearity and the possible existence of a threshold. As explained in the Biostatistics Core, this will be done using flexible modeling techniques such as penalized splines.

3. EXPECTED RESULTS, OUTCOMES
Results from this study will provide key information about the health effects of individual air pollutants, sources, and pollutant mixtures. By virtue of its size, detailed participant information, and the wealth of health outcome data we have already collected, the NAS cohort will continue to give us valuable insights, particularly from the extensions to cognitive data and Telomere length. This project will allow us to identify the sources, components and mixtures most relevant for the effects of air pollution on cardiovascular disease and cognition. In addition, we will determine the sources, components and mixtures most important to inflammation, endothelial function, and autonomic function, for both short-term and long-term exposure. It will also examine phenotypic, genetic, and social modifiers of all identified associations, and ascertain the shape of the dose-response curves. Innovative components include the availability of repeated cognitive function tests in each visit back to the early 1990s and the ability to prospectively examine effects of air pollution on telomere length changes over time. Moreover, there is extensive overlap in outcomes across the projects in our center. We will be able to confirm associations with cognitive performance in a replication cohort: The Framingham Offspring, and examine effects over the lifespan by comparison with the Viva and Framingham Generation 3 cohorts. Project 1 measurements of cerebral blood flow will also offer insight into potential mechanisms. Inflammatory and vascular responses will be replicated in Framingham Offspring, age-extended in Generation 3, and experimentally confirmed in Project 1.

3.1 Future opportunities: The opportunities created by this Center go far beyond the evaluation of these important outcomes. By expanding the exposure information to include short- and long-term measures of multiple pollutants and mixtures in a cohort with a large amount of data collection funded by other sources, and stored samples back to the 1990s, this study will also create important opportunities, as funds external to this proposal become available, to evaluate pollution effects on, and modification by:1) DNA methylation of critical pathways (e.g. NFKb, MAPK, Glucocorticoid receptor, Asthma pathways, Nrf2 mediated oxidative stress response); 2) Intima media thickness of the carotid artery (450 measurements so far); 3) Pulmonary function
tests and obstructive airway disease; 4) Electrocardiographic changes, including QT and PR interval, and Minnesota Codes; 5) Modification of effects by genetic polymorphisms along specific pathways; and 6) miRNA expression for miRNA that are associated with cardiovascular disease.

3.2 Benefits of an integrated approach: Since both cardiovascular and cognitive measures will be available in children (Project 4) and adults (Project 3) as well as the elderly, we will be able to examine consistency across the lifecourse. Finally, the availability of data from the Framingham Offspring cohort, which is only slightly younger than our participants, will provide built-in validation for many of our hypotheses. Any findings in this study will be replicated in the Framingham offspring cohort, and examined across the lifecourse in the Framingham generation 3 and Viva cohorts.

3.3 Better Protection of Public Health: The cognitive effects of air pollution exposure are not yet well understood, and with repeated measurements since the mid-1990s, we are well-positioned to study this. The elderly represent a particularly susceptible population, and the growth in the number and proportion of older adults in the United States is unprecedented. By 2030, the proportion of the U.S. population aged 65 and older will double to about 71 million older adults, or one in every five Americans. Cognitive decline in the elderly is also a growing burden. Recent estimates are that 6.4% of people over age 60 years in North America have dementia, with the number expected to almost triple by the year 2040. Importantly, small changes in cognition are strong predictors of eventual development of dementia. Previous studies have also reported that heterogeneity in cognition is especially pronounced in the elderly compared to younger adults. This raises the question of whether there are environmental causes of this heterogeneity. Air pollution exposure has been linked to increased inflammation in the brain, and brain inflammation has been implicated in the development of Alzheimer's disease. Other outcomes we propose to study, including blood pressure and vascular outcomes, are known to be predictors of heart attacks and mortality, making them important intermediate outcomes to study. Our novel exposure modeling will yield far better, more precise, estimates of the effects of air pollution on these outcomes by examining multi-pollutant mixtures, sources, and GIS-based individual exposures. In addition, the estimates of the effects of mixtures will help EPA regulations because we will not have to worry about pollutants being surrogates for other pollutants because we consider them all together, so we know we are regulating the most toxic pollutants.

4. GENERAL PROJECT INFORMATION
For this project we have brought together a team consisting of Investigators with international reputations and a history of collaboration. Dr. Mittleman is well known cardiovascular epidemiologist who has collaborated with Dr. Schwartz on other grants involving heart attacks and heart failure, as well as on studies involving the NAS. Dr. Bellinger is a world leading expert on the effects of environmental exposures on cognition, and has collaborated with Dr. Schwartz for more than a decade. Dr. Kubzanski, is an international expert on psychosocial stress. She has written an important methodologic paper on examining interactions with air pollution, collaborated with Dr. Schwartz on the interactive effect of stress and lead on cognition in the NAS, and published widely on the effects of stress in the NAS. Dr. Gold is a recognized epidemiologist who has collaborated with Dr. Schwartz for over 20 years. Dr. Zanobetti is well known for her statistical analysis of air pollution studies, and Dr. Vokonas is the PI of the NAS at the Veteran’s Administration.
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