ABSTRACT

The independent and joint effects of particles and gases on acute autonomic function and inflammation changes are poorly defined, especially when the particles and gases originate from the same source. To improve our understanding about pollutant mixtures and source-specific effects, we propose to use a crossover study (Project 2) to examine whether particulate and/or gaseous pollutants emitted from motor vehicles are associated with autonomic dysfunction and pulmonary and systemic inflammation. The study will test these hypotheses by exposing 36 older adults repeatedly either to particulate plus gaseous motor vehicle pollution or to only gaseous motor vehicle pollution. For both exposure scenarios, participants will be exposed through 5-hr long field trips via diesel-powered buses. Participants will include older adults who may be more sensitive to particulate pollution and will likely include individuals with coronary artery disease. Selected participants will live in or near retirement facilities located in suburban Boston. Project 2 will be conducted in Years 3 through 5 of the Center.

Field trips will occur in the fall and spring, when regional pollution is low, so that motor vehicle pollution comprises a larger fraction of particulate mass. Twelve subjects, randomly divided into two groups of six, will participate in each field trip, with groups riding on separate buses that will follow the same route. On one bus, the air will be unmodified, thus naturally exposing its riders to elevated concentrations of particles and gases from the bus and surrounding vehicles. Air in the second bus will be filtered to remove all particles. Thus, individuals traveling on the second bus will be exposed only to gaseous pollution, predominately from vehicles. Exposure scenarios for the groups will be alternated in a second field trip, which will occur one-to-two weeks later to prevent carryover of any effect from the first trip while minimizing differences in weather and ambient particle composition. Study participants will be randomized to the sequence of exposures to avoid bias. A total of 72 person-trips will be made (six groups and six trips).

Health and exposure monitoring will be identical to methods used in our precursor St. Louis Bus study, which was performed as part of our existing Center. Before, during, and after each trip, participants will be monitored for heart rate variability (HRV) as a measure of autonomic function, exhaled NO (eNO) as a measure of pulmonary inflammation, and blood markers as a measure of systemic inflammation. In addition, oxygen saturation and blood pressure will be measured repeatedly, as potentially inter-related intermediate markers of cardiac morbidity. Personal group-level measures of black carbon (BC) and particle counts (PC) will also be measured before, during and after each trip to assess exposures to mobile source pollution, along with measures of $PM_{2.5}$ and $PM_{2.5-10}$, ozone (O_3), carbon monoxide (CO), nitrogen oxide (NO), and total nitrogen oxides (NOx).

Data from this study will provide critical information about the roles of pollution mixtures and motor vehicle pollution in cardiac dysfunction. Through its crossover design and use of bus trips to elevate motor vehicle exposures, this study provides a novel, efficient and effective means to examine the combined and separate effects of particulate and gaseous motor vehicle pollution. The crossover design not only allows separation of the effects of gases from those of both particles and gases, but also allows these effects to be examined both by group and by individual. Furthermore, the use of bus trips to elevate exposures allows us to study "frail" older adults, who for ethical reasons cannot be examined in controlled human studies.

1. OBJECTIVES

This study will use a crossover design to test the following primary hypotheses:

- Mobile source-related pollution (particles and gases) will adversely affect:
  - Autonomic function as assessed by time and frequency domain measures of HRV; and
  - Pulmonary and systemic inflammation as measured using eNO, C-reactive protein (CRP), interleukin-6 (IL-6), fibrinogen, white blood cells (WBC) with differential, and for endothelial cells as measured using soluble intercellular adhesion molecule 1 (ICAM-1);
- Mobile source-related gaseous pollutants will adversely affect autonomic function and pulmonary and systemic inflammation, and;
- The risks posed by mobile source-related particulate and gaseous pollution are greater than those posed by gaseous pollution alone.

2. INTRODUCTION

2.1. Particles, Cardiovascular Risk, and Mobile Sources: Since the late 1980's, numerous studies have found particulate air pollutant concentrations to be responsible for excess mortality, as associations between particulate air pollution and daily deaths have been reported for cities in the US, Europe, Latin America, and Asia[1,2,3,4]. These associations have been shown to be independent of weather[5,6], season[7], and ambient gaseous air pollutant concentrations[4,8]. In recent epidemiological studies, these associations have further been demonstrated to be related specifically to daily deaths from cardiovascular disease[9] and have also been demonstrated for hospital admissions for myocardial infarctions and other heart conditions[10,11,12,13]. The particulate constituent(s) and sources responsible for the observed effects are not known; however, attention has focused increasingly on particles from motor vehicles, which fall mostly within the fine ($PM_{2.5}$) and ultra-fine (<0.1 μm) size range[14]. This attention has resulted from findings from epidemiological studies that showed greater health risks for particles associated with motor vehicle emissions. In our analysis of elemental concentrations in Six-US Cities, for example, particles from mobile sources were shown to impact daily mortality rates (particularly for cardiovascular deaths) more strongly as compared to particles from other sources[15]; similarly, epidemiological study findings in the Netherlands[16] and other Western European cities[17] suggest that traffic particles are associated with an increase in mortality. More recently, in an analysis performed as part of our existing Harvard/EPA PM Center, the increased risk of cardiovascular hospitalization associated with a fixed increment in exposure to airborne particles increased with the percent of the particles from traffic sources[18].

2.2. Traffic-Related Effects on Autonomic, Inflammation and Vascular Outcomes: Results from epidemiological and animal studies suggest several mechanisms by which traffic-related particles may damage cardiac health. In our early panel studies, for example, we showed that ambient $PM_{2.5}$ and O_3 concentrations produced reductions in heart rate variability (HRV) (r-MSSD), a known risk factor for sudden death[19,20,21,22,23,24,25,26], in older adults[27] and in a younger occupational cohort[28] in Boston. Correspondingly, we found ambient NO_2 and $PM_{2.5}$ concentrations to be associated with defibrillator discharges due to ventricular arrhythmias in patients with implanted cardioverter defibrillators[46]. Our more recent Center-funded studies have shown black carbon (BC), a marker of traffic, to have the strongest effects on heart rate variability. In our summer 1999 panel study of older adults, we found BC to be more strongly associated with reduced HRV than non-traffic particles[29]. CO, a gaseous pollutant emitted outdoors primarily by motor vehicles, showed similar patterns of association as BC, although associations disappeared after controlling for BC. In addition, we found that an elevated BC level 5 hr prior to testing was associated with a 0.11 mm (p=0.001) ST-segment depression in continuous models, and 10.4 times the risk of ST-segment depression of $\geq$0.5 mm (p=0.03) in dichotomous models. In contrast, no significant relation of $PM_{2.5}$ to ST-segment depression was found. CO was not a confounder of this association. For subjects with prior myocardial infarctions, the effect of BC on reduced SDNN was almost four times as great. These findings suggest that traffic-related particles harm the cardiovascular system by compromising autonomic control of the heart.

The mechanisms by which particles may perturb autonomic function are not known; however, Seaton *et al.*[30,31] proposed that particles may do so by initially increasing pulmonary inflammation, for which NO concentrations in expired air represents a noninvasive measure[32]. Exhaled NO (eNO) concentrations are typically elevated in individuals with chronic cough[33], chronic obstructive pulmonary disease (COPD)[34] and asthma[35], as a result of increased pro-inflammatory cytokines which stimulate endogenous NO production through activation of inducible NOS (iNOS)[36]. Increased levels of eNO have recently been linked to traffic-related pollutant concentrations. In a study by Van Amsterdam *et al*.[37], morning ambient air pollution levels were compared to eNO levels on 14 consecutive workdays in 16 non-smoking adults. The strongest associations were found with ambient NO and CO, which are often used as a tracer of motor vehicle pollution. Correspondingly, in a longitudinal study of elementary school children[38], PM_{10} and the traffic markers black smoke, NO, and NO_x were associated with increased eNO, reduction in peak expiratory flow and increases in inflammatory markers in nasal lavage samples. Associations between increased eNO and $PM_{2.5}$ were also observed in a large panel study of children in Seattle[39] and in our EPA Center-funded panel study of 29 non-smoking older adults in Steubenville, OH[40], indicating that air pollution may result in acute changes in pulmonary inflammation[40].

It is possible that BC and other traffic related particles are also associated with later steps in the causal pathway for particles, such as systemic inflammation followed by vascular dysfunction. Markers of systemic inflammation and vascular dysfunction include CRP, IL-6, sICAM-1, and von Willibrand's factor (VWF), each of which have been shown to be independently and jointly associated with increased cardiac risk[41,42,43,44]. Similarly, an increasing number of studies have shown air pollution to increase levels of these and other systemic inflammation markers. For example, we found that acute elevation in ambient particle levels were associated with an elevation in fibrinogen in a large epidemiological study,[45] with this effect strongest in participants with chronic obstructive lung disease. Peters and coworkers[46] 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[47] and animal studies[48] of urban particles. In addition, in a London study, black smoke and NO_2 showed stronger associations with plasma fibrinogen – an intermediate marker of cardiovascular disease than PM_{10}[49]. Correspondingly, a recent controlled human exposure study found that exposure to diesel particles for one hour at 300 $\mu g/m^3$ resulted in increased levels of peripheral neutrophils, and increased levels of vascular cell adhesion molecule 1 (VCAM) and ICAM[50].

Other studies have examined the relationship between air pollution and vascular dysfunction using blood pressure as a measure of vascular reactivity. Higher levels of air pollution were found to predict higher blood pressure in a Los Angeles panel study[51] and a large cross-sectional German study[52]. Similarly, Zanobetti *et al.*[53] found a 2.8% increase (2.8 mm Hg) in resting systolic blood pressure per 10.5 ug/m^3 of ambient $PM_{2.5}$ during the five days prior to the measurement in patients with pre-existing cardiovascular disease. Associations between 2-day mean ambient $PM_{2.5}$ and increased diastolic blood pressure (10.2% or 7.0 mm Hg) and resting mean arterial blood pressure (5.2% or 4.7 mm Hg) were also found during exercise in persons with high resting heart rates ($\geq$ 70bpm). The small pollution-related increases in blood pressure observed in these studies could reflect a systemic effect, including a coronary artery increase in tone, which would suggest an increased risk of plaque rupture and cardiac ischemia[54,55]. Alternatively, the effects of particles on vascular tone may be mediated through an increase in systemic inflammation, and, as suggested by Brook and colleagues[56], consequent increased vascular endothelin expression by direct mechanisms or via oxidative stress pathways.

2.3. Separation of Traffic-Related Particle Effects from Traffic-Related Gaseous Effects: Since concentrations of traffic-related gaseous pollutants such as CO and NO_2 tend to be strongly correlated with BC and other traffic-related particle concentrations, it is possible for the observed PM effects to be confounded by the gaseous co-pollutants. The extent to which confounding may occur depends in large part on the ability of CO and NO_2 to elicit deleterious effects on autonomic dysfunction, pulmonary and systemic inflammation independently or in conjunction with particles. Both CO and NO_2 have been linked in epidemiological studies to a variety of adverse outcomes, with CO more often associated with cardiovascular effects and NO_2 with respiratory effects. For example, NO_2 was associated with defibrillator discharges due to ventricular arrhythmias[46] and increased plasma fibrinogen levels[57], while both ambient NO and CO were linked to higher eNO levels[58]. Furthermore, in the APHEA study, NO_2 was shown to be both a confounder and a modifier of PM risks, as (1) NO_2 was found to reduce PM risk estimates by approximately 50% in multi-pollutant models and (2) cities with higher NO_2 levels showed larger PM effects[59,60]. Observed effects of both CO and NO_2 may be stronger in individuals with pre-existing heart or respiratory disease. The observed adverse effects of CO and NO_2 and their strong correlations with traffic-related particle concentrations raise serious concerns about the potential for confounding of PM effects.

In addition, CO, NO_2 and other gaseous pollutant exposures may enhance or possibly diminish the effects of particulate exposures. In our EPA Center-funded studies of rats with myocardial infarctions (MI), we found CO exposures and concentrated ambient particle exposures had opposite effects. CO (35 ppm) reduced ventricular premature beat (VPB) frequency significantly during the exposure period as compared to controls, while concentrated ambient particles (CAPs) exposure showed a positive impact on VPB frequency[61]. These results, suggesting that gases and PM may have opposing effects on cardiac health, underscore the importance of differentiating the effects of PM and gases in humans.

In epidemiological studies, however, the independent or combined effects of gaseous and particulate pollutants have been difficult to study. Epidemiological studies traditionally have relied on statistical approaches based on single or multiple pollutant models to examine multiple pollutant effects. This approach has substantial limitations, especially when particles and gases are derived from the same source. As a result, different approaches are needed in epidemiological studies to examine the separate and joint effects of particles and gases.

2.4. Spatial Heterogeneity in Traffic-Related Particle Concentrations: Studies of traffic-related particles conducted to date 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[62,63,64]. In a study conducted in Harlem, New York City, EC concentrations on four geographically distinct sidewalks varied 4-fold between sites, while mean $PM_{2.5}$ 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[64]. Similar results have been reported in European studies, including the Small Area Variation in Air Quality and Health (SAVIAH) study in the Netherlands[63]. Spatial variation in traffic-related particle concentrations is supported by results from our recent Center-funded exposure study of 28 Boston area homes. In this study, we found 24-hr EC concentrations at the homes to be only moderately correlated with concentrations measured at our stationary ambient monitoring site located in downtown Boston. Home-specific Spearman correlation coefficients for comparisons between home and ambient EC concentrations had a median value of only 0.56, as compared to median correlation coefficients for SO_4^{2-}, a regional pollutant, of 0.95. Home-specific correlation coefficients for EC also ranged substantially by home (25-75%: 0.32-0.72). Both the low median correlation coefficient and the wide range in home-specific coefficients indicate that spatial variation in outdoor EC concentrations is substantial, likely as the result of EC emitted by motor vehicles.

As a result of this spatial variation and also of their low and varying penetration efficiencies[8], ambient concentrations have also been shown to be weak proxies of 24-hr exposures to EC. In our Center-funded Boston panel study, the median subject-specific Spearman's correlation coefficient between personal EC exposure and ambient EC concentrations during the winter was 0.41, approximately half that for SO_4^{2-} (r_s=0.82)[65]. On average for a group of individuals, however, 24-hr ambient EC concentrations are generally associated with corresponding personal exposures, albeit with substantially more error. This error is even greater for periods less than 24-hr, particularly when individuals spend time in motor vehicles or other microenvironments with significant local sources[66].

2.5. Examination of Traffic-Related Effects on Cardiovascular Health: For short term effects, it has been difficult to examine the health effects of traffic-related particles using traditional epidemiological study designs, which rely on 24-hr ambient pollutant concentrations measured at stationary ambient monitoring (SAM) sites to estimate exposures for study populations. Existing EPA compliance monitoring networks only measure ambient PM_{10} and $PM_{2.5}$ concentrations, both of which are not specific indicators of motor vehicle emissions. Although central site monitoring of BC and other traffic-related pollutants is sufficient to estimate average exposures for a population, it is an imprecise measure of traffic-related exposures for an individual, especially over periods of an hour or less, since ambient concentrations of traffic particles, such as BC and PC, vary temporally over short time-periods, spatially over short distances, and by micro-environment.

To minimize these limitations, we used a novel exposure strategy to examine the cardiovascular effects of traffic-related pollution in St. Louis, MO. In this study we used HRV, blood pressure, eNO, systemic inflammatory markers, and corresponding personal particulate markers for a cohort of 44 elderly individuals, as they traveled on repeated field trips aboard a diesel-powered bus. By taking participants on field trips by bus, we increased the concentration of and variability in their traffic-related particulate and gaseous exposures and reduced correlations between traffic- and non-traffic pollutants. Furthermore, by measuring personal particulate exposures continuously for the group of participants, we were able to account for spatial variability in traffic-related particle concentrations and as a result, to minimize exposure measurement error.

In our proposed PM Center, we plan to perform a follow-up Bus study in Boston during Years 3-5 of the Center. This follow-up study will enable us to separate the effects of mobile source-related particulate and gaseous exposures on autonomic and inflammatory outcomes in 36 elderly individuals. As in St. Louis, this study will use bus trips to elevate participants' exposures to motor vehicle pollution and will measure cardiovascular health indicators and personal group-level exposures to motor vehicle and other pollutants using the previously established methods. The proposed study, however, will differ in three important aspects. First, study participants will be selected to include "frail" individuals who may be particularly sensitive to particle exposures, as defined based on Project 1 (NAS study) results. Second, exposures will be administered following a crossover design in which participants are randomized repeatedly to be exposed either to particulate and gaseous motor vehicle pollution or to only gaseous motor vehicle pollution. Participants in both groups will be exposed simultaneously using two different buses. Through this crossover design, Project 2 will maximize variability in motor vehicle-related exposures and will allow us to examine the joint and independent effects of particle and gaseous pollution on cardiovascular health. Third, the study design mimics that of our controlled human (Project 3) and animal studies (Project 4). The proposed study has the advantage of exposing relatively susceptible individuals in groups as opposed to previous investigations that expose healthy individuals one at a time.

3. PRELIMINARY STUDIES

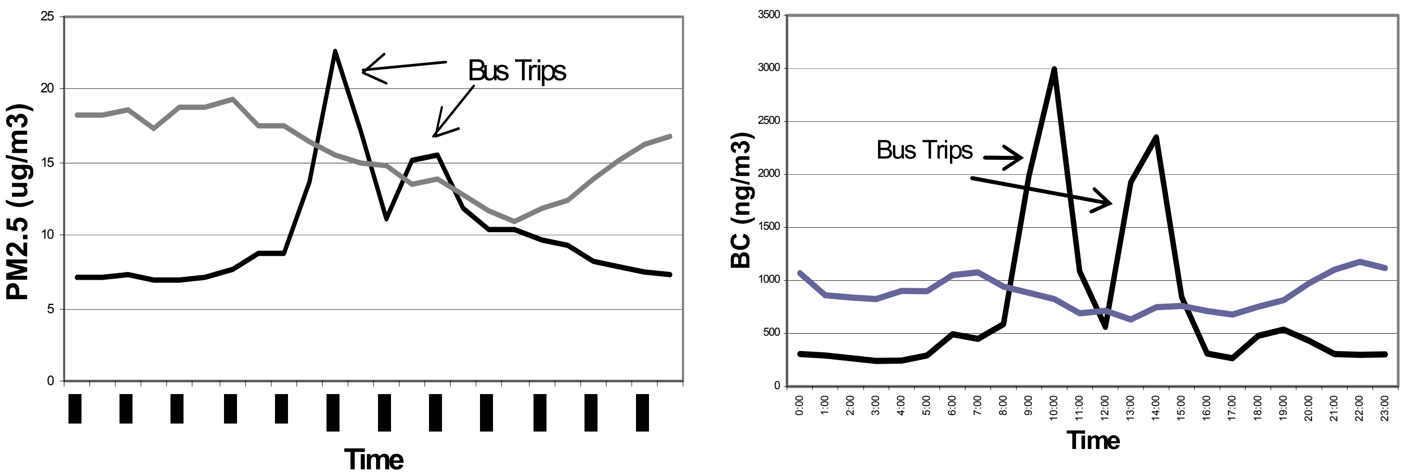
The proposed Project 2 continues our efforts to characterize the effects of particle exposures on cardiovascular health by focusing on the joint and independent effects of particles and gases from a single source type. In this effort, we have performed numerous panel studies of individuals sensitive to particle exposures in several cities, including Boston, MA, Atlanta, GA, Steubenville, OH, and St. Louis, MO, with most of these studies conducted in part through our existing Harvard/EPA PM Center. While differing in specific objectives and study design, each of our panel studies has successfully combined sophisticated exposure and health measurements to examine relationships between particulate exposures and a variety of indicators of autonomic dysfunction, pulmonary and systemic inflammation, and vascular dysfunction. To date, our panel studies have demonstrated relationships between ambient $PM_{2.5}$ concentrations and decrements in HRV, ST-segment depression, and increased defibrillator discharges, blood fibrinogen levels, eNO levels, and blood pressure, with the most recent studies of HRV and ST-segment depression showing the strongest effects for traffic-related BC concentrations.

The proposed Project 2 will focus specifically on the effects of traffic-related pollution and builds on the design of our St. Louis Bus study, which was conducted as part of our EPA Particle Center. In St. Louis, we monitored the cardiovascular health of 44 individuals living in retirement facilities as they traveled on field trips aboard a single, diesel-powered bus. Markers of altered cardiovascular function including HRV, heart rate, inflammatory indicators in the blood, and oxygen saturation of the blood were measured for participants during four separate 24-hr periods as the individuals traveled between the health testing room, a moving shuttle bus, indoor locations within the city, and his or her senior residence facility. Micro-environmental

$PM_{2.5}$, BC and fine particle count ($PC_{0.3\text{-}1.0}$) exposures were assessed continuously for the study participants using two portable monitoring carts that traveled with the study participants throughout the day. Ambient concentrations were obtained from the US EPA-funded St. Louis Super SAM site.

3.5.1. Exposures Measured in the St Louis Bus Study: As shown in Figure 1, personal exposures to $PM_{2.5}$, BC and $PC_{0.3\text{-}1.0}$ were significantly higher when participants were aboard the diesel-powered shuttle bus as compared to when they were in their residence facilities ($p<0.001$). Exposures were the most elevated for BC. It can be assumed that elevated exposures during bus trips were attributed to emissions from surrounding vehicles and the shuttle bus, since mean concentrations at the SAM site during the bus and facility periods were comparable.

Figure 1. Hourly Micro-environmental* and Ambient $PM_{2.5}$ and BC Concentrations



*Microenvironmental $PM_{2.5}$ (left) and BC (right) exposures were measured as participants traveled on a field trip via two bus rides and spent time in their residence facility. Micro-environmental exposures are shown in black; ambient concentrations are shown in grey. Exposures and concentrations averaged by hour.

3.5.2. Preliminary findings – Exhaled NO: We found eNO concentrations collected prior to participation in a bus trip to be significantly associated with $PM_{2.5}$ and $PC_{0.3\text{-}1.0}$ averaged over the previous 24-hrs (Table 1). For example, an inter-quartile increase in the 24-hr mean ambient $PM_{2.5}$ of 10 $\mu g/m^3$ resulted in a 15% (95%CI: 6 – 26%) increase in eNO using linear models adjusted for day of week, ambient apparent temperature, past nitrate consumption, recent meal, time between sample collection and analysis, study room NO concentrations, and a random subject effect. A similar increase for personal $PM_{2.5}$ (as measured by the portable monitoring carts inside the facility) corresponded to a 20% (95%CI: 1 – 43%) increase in eNO, while an inter-quartile (IQR) change in $PC_{0.3\text{-}1.0}$ of about 70 pt/cc resulted in 30% increase in eNO (95%CI: 1 – 43%). Changes in BC, CO, NO, and NO_2 were not significantly associated with deviations in eNO at the 95% confidence level.

On the day following the bus trip, we found similar effect estimates for measures of micro-environmental $PM_{2.5}$ (20%, CI: 6-35%) and $PC_{0.3\text{-}1.0}$ (23%, CI: 8-40%) when identical models were used. While ambient $PM_{2.5}$ was predictive of eNO when participants were at their living facilities for the previous 24-hr, ambient $PM_{2.5}$ was not predictive of eNO when the same individuals took part in a field trip that included two hours on the highway. While the gases remained non-predictive of post-trip eNO, BC became a significant predictor of eNO (20%, CI: 2-40%) for samples collected on the days after the bus trips. Data suggest that elevated exposures to traffic-related particles result in increased pulmonary inflammation as measured by eNO. Future findings will refine our analyses of the effects of motor vehicle exposures on eNO and determine whether autonomic effects such as HRV, ST-segment depression, and arrhythmias are also associated with motor vehicle exposures.

Table 1. Effect estimates (per IQR) for pre- and post-trip exhaled NO and air pollution

	Location	PRE-TRIP				POST-TRIP			
		df	**Estimate[a]**	**LCL**	**UCL**	**df**	**Estimate[a]**	**LCL**	**UCL**
$PM_{2.5}$	micro	**81**	**1.20**	**1.01**	**1.43**	**85**	**1.20**	**1.06**	**1.35**
	ambient	**90**	**1.15**	**1.06**	**1.26**	91	1.02	0.91	1.14
BC	micro	80	1.03	0.84	1.27	**91**	**1.20**	**1.02**	**1.40**
	ambient	90	1.07	0.96	1.20	91	1.09	0.94	1.27
PC fine	micro	**80**	**1.33**	**1.10**	**1.61**	**83**	**1.23**	**1.08**	**1.40**
CO	ambient	77	0.93	0.85	1.02	76	0.95	0.85	1.06
NO	ambient	74	1.04	0.96	1.13	69	1.05	0.95	1.15
NO2	ambient	74	0.95	0.82	1.10	69	1.15	0.93	1.42

[a] Estimates were calculated using mixed-effects linear models with random intercepts and adjusting for day of week, ambient apparent temperature, past nitrate consumption, recent meal, time between sample collection and analysis, and room NO concentrations. Effect estimates are for approximate IQR differences of 10 $\mu g/m^3$, 500 ng/m^3, 70 pt/cc, 0.25 ppm, 11 ppb, and 8 ppb for $PM_{2.5}$, BC, PC fine, CO, NO, and NO_2, respectively.

3.5.3. Preliminary findings – Systemic Inflammation: In general, higher (albeit statistically non-significant) inflammation was observed among individuals with symptoms of metabolic syndrome, which was defined to include hypertension, diabetes, and obesity. When the entire population was included in the analyses, changes in inflammation marker levels were not associated with any pollution metrics. For the four individuals with all three symptoms of metabolic syndrome, positive associations between air pollution and inflammatory markers were consistently observed (Table 2).

Effects were generally greatest for the previous 2 hours of exposure, with effects declining at longer moving averages. Only CRP remained elevated over longer averaging periods, as might be expected based on its physiology. When analyses were performed for individuals with varying degrees of metabolic syndrome, the resulting dose-response relationship appears to confirm that increasing symptoms of metabolic syndrome increase susceptibility to the inflammatory effects of ambient PM (Figure 2).

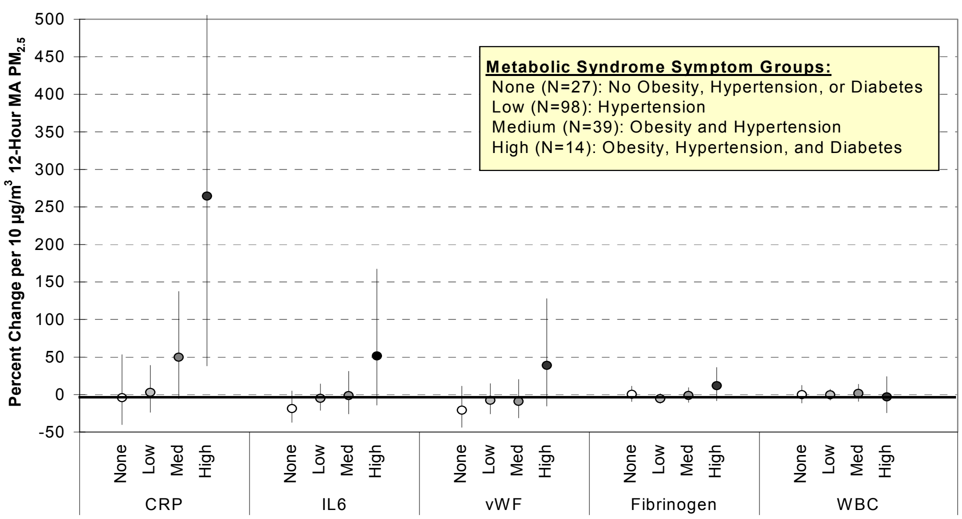
With the other pollutants, significant positive associations were also found with CRP only among metabolic syndrome individuals (Table 2). The largest effects were found for $PM_{2.5}$ and BC, with an approximate 2-fold increase in CRP per IQR change in pollution. O_3 and PC also elevated CRP, while ambient CO and NO had negative associations with CRP. Although the gases and micro-environmental particles were negatively correlated, the relationships were weak (i.e. less than 30%). When ambient concentrations were used as the exposure variable, the observed effect estimates were lower than those found for micro-environmental exposures. These lower effect estimates likely result from increased measurement error in the ambient data, which do not account for personal activities.

Table 2. Effect Estimates for CRP by Metabolic Syndrome

Pollutant (48-Hour MA)		MICROENVIRONMENT		AMBIENT	
		No Metabolic	Metabolic	No Metabolic	Metabolic
$PM_{2.5}$	% Change per 10 μg/m^3 [95% CI]	-10 [-32, 19]	**148 [39, 343]**	1.0 [-15, 20]	**105 [21, 245]**
	N	114	14	121	14
	p-value	0.47	0.0029	0.58	0.0086
BC	% Change per 500 ng/m^3 [95% CI]	-16 [-35, 8.8]	**113 [16, 291]**	0.87 [-19, 25]	**88 [10, 220]**
	N	121	14	121	14
	p-value	0.57	0.016	0.94	0.023
PC	% Change per 30 pt/cc [95% CI]	-2.5 [-11, 6.9]	**42 [15, 74]**		
	N	117	13	NA	NA
	p-value	0.59	0.0015		
CO	% Change per 0.2 ppm [95% CI]			1.4 [-22, 18]	**-35 [-51, -14]**
	N	NA	NA	109	14
	p-value			0.85	0.0043
NO	% Change per 10 ppb [95% CI]			8.9 [-6.0, 26]	**-68 [-99, -22]**
	N	NA	NA	96	12
	p-value			0.26	0.032
O_3	% Change per 12.5ppb [95% CI]			1.1 [-16, 17]	**50 [9.0, 105]**
	N	NA	NA	111	14
	p-value			0.90	0.015

NA = Data not available for this location.

Figure 2. Effect Modification by Metabolic Syndrome Symptoms



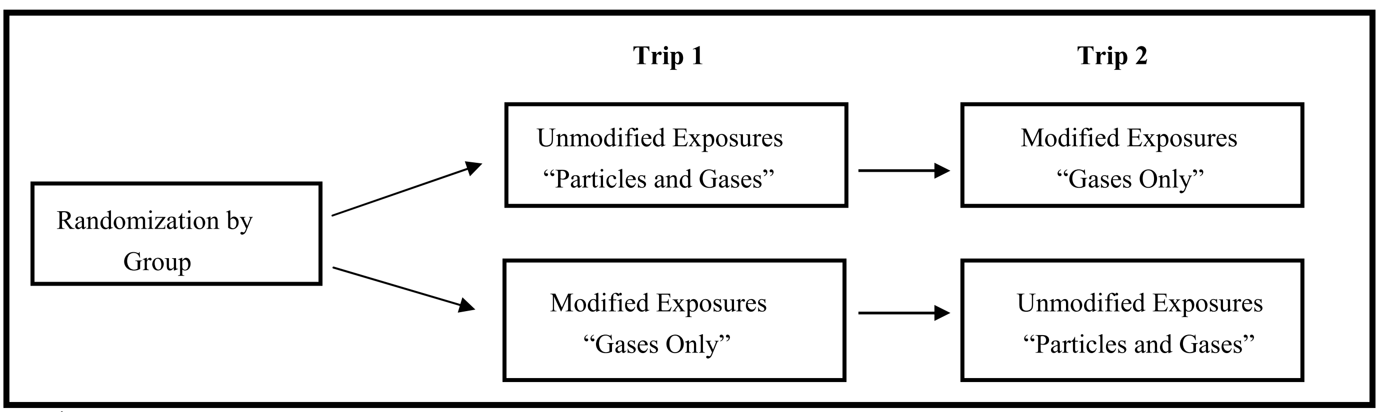
These results suggest that air pollution exposures are associated with systemic inflammation among seniors having at least one symptom of metabolic syndrome, suggesting pollution impacts for a large proportion of the elderly in the U.S. (approximately 33% with obesity and 50% with hypertension). Inflammation associated with air pollution appears to occur acutely, with most effects within the first day of exposure.

4. APPROACH

4.1. Study Design: We will assess the relationship between traffic-related pollutant exposures and autonomic, vascular, and inflammatory outcomes using a cross-over design, with data used to test whether motor vehicle related effects differ for particles and gases. The study will be conducted during Years 3 through 5 of the Center. [Note that health endpoints may be modified if findings from our earlier Center studies warrant such changes.]

4.2. Sample Selection and Study Protocol: Participants will include older adults who are sensitive to particulate pollution, likely including individuals with coronary artery disease who live in senior citizen residence facilities. If possible, individuals from the NAS cohort that live near one of these residence facilities may also be asked to participate. Thirty-six older adults will be exposed repeatedly either to particulate plus gaseous motor vehicle pollution or to only gaseous motor vehicle pollution as they travel on 5-hr long field trips via diesel-powered buses. Twelve older adults, randomly divided into two groups of six, will participate in each field trip, with groups riding on separate buses that will follow each other throughout each trip (Figure 4). On one bus, the air will be unmodified, thus naturally exposing its riders to elevated concentrations of particles and gases from the bus and surrounding vehicles. Air in the second bus will be filtered to remove all particles. Thus, individuals traveling on the second bus will be exposed only to gaseous pollution, predominately from vehicles. Exposure scenarios for the groups will be alternated in a second field trip, which will occur one-to-two weeks later to prevent carryover of any effect from the first trip while minimizing differences in weather and ambient particle composition. Study participants will be randomized to the sequence of exposures to avoid bias.

Figure 4. Exposure Plan: By Field Trip[*]



[*]Two groups of six individuals go out on each field trip, with exposure scenarios randomized by group. In total, three sets of two groups will be randomized, for a total of six trips and six groups.

A total of 12 field trips (6 groups x 2 trips/group) or 72 person-trips will be made as part of the study. Field trips will occur in Year 4 during the fall and spring, when regional pollution is low so that motor vehicle pollution comprises a larger fraction of particulate mass. Field trips are intended to increase exposures to diesel or traffic particles for five hours. As such, trips are likely to include activities in which exposures to mobile sources will occur, such as bus tours of New Hampshire, downtown Boston, or historic Concord and Lexington.

4.2.1. Participant Recruitment and Selection: Subjects will be recruited from individuals living in senior citizen residence facilities located in suburban Boston and away from major highways (to reduce participants' background exposures to motor vehicle related pollution).

Managers of senior residence facilities located in Boston area suburbs will be contacted via letter, followed by a telephone call. During this call, the study purpose, participant profiles, and requirements will be explained. Managers will be specifically asked for permission to contact residents through posted flyers and presentations and to provide a room for health assessments. Individuals living in facilities that express interest will then be identified through posted flyers and presentations. Individuals who express interest will be contacted by telephone, when a brief pre-screening questionnaire that will address inclusion and exclusion criteria and logistical issues will be administered. If the candidate meets our initial criteria, a screening visit appointment will be scheduled. At this visit, the individual will be enrolled in the study and their written consent for participation will be obtained.

The study cohort will include individuals greater than 55 years of age, both men and women, and individuals of any race. Subjects will be selected for inclusion based on the same criteria used in our earlier St. Louis bus study, including ability to board the bus and navigate short flights of stairs unassisted, non-smoking status (for at least the prior six months), willingness to participate in the study, and cardiovascular health. Individuals will be excluded from participation in the study if during the screening evaluation it is determined that (see below) they have congestive heart failure, atrial fibrillation, atrial flutter, and/or paced rhythm. In addition, individuals with left bundle branch block will be excluded from the study, since an adequate measure of HRV cannot be ascertained.

4.2.2. Screening: Prior to enrollment in this study, all volunteers will participate in a screening evaluation during which they will complete a questionnaire regarding general health and housing situation. Measurements of height, weight, blood pressure, blood oxygen levels, and heart rate will also be recorded. Baseline pulmonary (via spirometry) and cardiovascular health (via a 12 lead ECG) evaluations will also be conducted. In addition, a baseline blood sample will also be drawn. This information will help to identify any medical (e.g., the use of a pace maker) or living (e.g., lives in a smoking household) conditions that may interfere with data collection. The systolic and diastolic blood pressure readings will be recorded to the nearest 2 mm Hg as the mean of two measurements with the subject seated. Informed Consent will be gathered before any testing takes place. Consent will also be obtained by fax or mail to the primary care physician, should there be findings during the testing that are of concern to the staff or that fit the criteria listed below. For example, if blood pressure exceeds 140/90, the subject will be advised to have his/her blood pressure rechecked by his/her nurse or physician. If the blood pressure exceeds 180/100, the subject will be advised to follow-up with his/her physician. All subjects will be provided with their blood pressure, oximetry, and ECG measurements on screening. Based on our experience in previous studies, we estimate that two to three times as many individuals will be screened as will enter into the study. If the subject meets the inclusion requirements after the pre-screening, subjects will be scheduled for their two field trips.

4.3. Health Measurements: Following selection for the study, each subject will be monitored during two field trips, in groups of six. Two groups of six participants will travel on each field trip (Table 3). On the morning before each trip, these participants will be administered a brief health questionnaire regarding chest pain, doctor visits, hospital visits, food intake, medication changes and whether the medication had been taken that morning. Five electrode leads will then be attached to the participant and connected to a Holter monitor for 24-hr collection. The participant will then lie quietly for ten minutes of rest. The ECG pattern on the Reynolds Holter during the rest period will be compared with the screening and other previously recorded ECG readings to ensure proper placement of the electrodes. The systolic and diastolic blood pressure readings will be recorded to the nearest 2 mm Hg as the mean of three measurements (see below) with the subject seated. A battery operated oxygen saturation monitor will be attached, with continuous monitoring for 3 minutes with printouts every 30-seconds of oxygen saturation. At this point, eNO measurements will be taken as detailed below. A study physician (e.g., Dr. Gold or Stone) will be on call to respond to questions regarding participant symptoms or blood pressure measurements prompting questions as to whether participants are in sufficiently good health to participate in the trip. [A manual of operations will be available to describe parameters that prompt technician calls to the study physician.] Participants (with their Holter monitors) will then board the bus for travel on their field trip. At the end of the bus trip and prior to each change in activity on the field trip, participants will be asked to sit quietly for ten minutes to obtain a resting ECG measurement. At the end of each bus trip, three resting blood pressure measurements will again be made in the health clinic room. Participants will then be allowed to leave the clinic room and go about their normal activities while continuing to wear the Holter monitor until their "post-trip" visit the next morning. At their post-trip visit, a questionnaire will be administered, three resting blood pressure measurements will be made, an eNO sample will be collected, and the Holter monitor will be removed. If at any time during monitoring, the participant experiences acute symptoms of angina or any other symptoms of clinical concern, Dr. Gold or Dr. Stone will immediately communicate with the primary care physician, with further testing for this participant stopped. In addition to the trip and lunch, participants will be compensated $40 per trip for their time.

After each post-trip visit, the CD/DVD containing Holter information will be brought to the Harvard AECG laboratory, where they will be downloaded and then analyzed. Collected blood samples will be iced and brought immediately to the Channing Laboratories for preparation, storage and analysis. eNO samples will be brought to HSPH laboratories immediately after their collection for analysis. Over the two study years, a total of 72 Holter CD/DVDs will be collected (2 CD/DVD/person), 216 sets of blood pressure measurements (2 pre-trip and 4 post-trip measurement sets/ person), 144 eNO samples will be collected (2 pre-trip and 2 post-trip measurement sets/person), and 72 blood samples will be collected (2 samples/person).

Table 3. Trip Schedule

Day	Data Collection	Monitor Location	Start Time
Pre-Trip	Begin Exposure Monitoring (RA)[1]	Residence Facility	8:00 AM
Trip	Two groups (of 6 participants each) enter health room - Questionnaire administration - Exhaled NO, resting BP measurements - Holter placement and monitoring	Health Room	8:00 AM
	Field Trip (including bus rides and lunch)[2]	Bus	9:00 AM
	Resting BP measurement[2]	Bus	1:30 PM
	Groups A and B return to normal activities[1]	Residence	1:45 PM
Post-Trip	Groups return to health assessment room[1] - Questionnaire administration - Exhaled NO, resting BP measurements - Removal of Holter monitor - Collection of blood sample	Health Room	8:00 AM

[1] Monitoring carts will be placed in a central facility location or the health room and will include instruments to measure BC, PC, $PM_{2.5}$, temperature, and relative humidity. [2] Carts will follow participants during field trip, including lunch. CO and NOx concentrations will also be measured during bus trips. Possible activities include bus tours of New Hampshire foliage, and historic Concord and Lexington.

4.3.1. Anthropometric measurements: Weight and height will be obtained for each participant. BMI will be calculated according to standard procedures.

4.3.2. Blood specimens: A blood specimen of approximately 30 ml at screening and 20 ml at each follow-up visit will be drawn from each participant from an antecubital vein without venous stasis. Care will be taken to avoid effects of injury to the vessel wall. Plasma will be aliquotted and stored at the Channing Lab, for batch measurement at the GCRC of IL-6 (high sensitivity) and sICAM (R&D Systems Minneapolis, Minnesota), both of which will be measured in plasma in duplicate using the enzyme-linked immunosorbent assay method. The concentration of CRP and fibrinogen will be determined using an immunoturbidimetric assay on the Hitachi 911 analyzer (Roche Diagnostics - Indianapolis, IN).

4.3.3. Holter Monitoring: Calibration of the Holter monitor will be initiated approximately 15 minutes prior to placing the electrodes. Holter cables will be attached to the electrodes with a stress loop created to avoid loss of contact. Skin preparation will include shaving, if necessary, alcohol wipe, and skin abrasion at the site of the electrode placement. Placement will be in a standard modified V5 and a VF position. Monitoring will be performed throughout each field trip and will continue for approximately 18 hours after the end of each trip.

4.3.4. HRV Measurements: When the CD/DVD has been received at the Harvard AECG Laboratory, the data will be transferred to the hard drive of the Del Mar/Reynolds Pathfinder Holter computer for analysis. Each recording will be edited for artifact and beat classification and then analyzed using 5-min epochs and using the entire recording as one epoch. Data will be analyzed for HRV, arrhythmia counts, ST-segment baseline values, and ST-segment deviation, using standard, validated algorithms. Time domain HRV outcomes will include (1) the standard deviation of normal RR intervals (SDNN); (2) the square root of the mean of the squared differences between adjacent normal RR intervals (r-MSSD), and the number of pairs of adjacent NN intervals differing by more than 50 ms divided by the total number of all NN intervals (PNN50). Frequency domain HRV outcomes will include high frequency (HF), low frequency (LF), and the LF/HF ratio, which will be used to reflect the balance between the sympathetic and parasympathetic components of the autonomic nervous system. A hard copy report of Holter data will be generated and stored in a dry safe, locked storage room. Holter endpoint data will be stored electronically and will be transferred electronically to the Data Coordinating Center for inclusion in the study dataset. The analyzed data will also be archived in electronic format, using a CD/DVD sent to the Data Coordinating Center for storage, a CD/DVD for storage offsite at a secure location and a CD/DVD at the AECG Core Laboratory. The Holter technician will use Reynolds software tools to process the ECG data under the training and supervision of Dr. Stone, with input from Dr. Gold. Exclusion criteria for HRV analysis will include unstable angina, atrial flutter, atrial fibrillation, and paced rhythm. Regions of noise and artifact will be eliminated. After correction, only normal-to-normal (NN) intervals between 150 and 5000 ms with NN ratios between 0.8 and 1.2 will be included for HRV analysis.

4.3.5. Blood Pressure and Oximetry: The automatic blood pressure machine (Welch Allyn Vital Signs monitor) and the oximeter (Nellcor) will be used to measure blood pressure and blood oxygen levels, respectively. Instruments will be calibrated at the manufacturer before the start of the study.

4.3.6. Collection and Analysis of eNO Samples: The sampling procedures will follow guidelines published by the American Thoracic Society for the collection of breath samples for off-line nitric oxide analysis[40,67]. The breath sample collection device will be assembled from standard mouthpieces and fittings used for spirometry and one-way valves to separate inspiratory and expiratory flows[68]. Due to the potential for contamination of breath samples with ambient NO, inspiratory air will be scrubbed using a NO filter consisting of Purafil® followed by activated carbon. Dead space air will be eliminated before collecting alveolar air by expiring through a one-way valve. In addition, a grab sample of air in the study room will be collected to allow for later analysis of the influence of the study room concentrations. Breath and room air samples will be analyzed for NO using a Thermoenvironmental Model42C Chemiluminescence NO_x Analyzer at HSPH.

4.4. Exposure Measurements: Motor vehicle exhaust exposures will be monitored on two buses, which will follow each other during each field trip. The bus for which exposures will be modified to remove all particles is already owned by the Center, while the second bus for which exposures will be unmodified (thus exposing participants to both particles and gases) will be leased for the study. Detailed information about the buses and particle removal technology is contained in the Technology and Monitoring Core; Section on "Mobile Particle Exposure Systems (MPES)."

Traffic-related and ambient air pollution will be measured continuously on the bus as well as 24-hr prior to and after each field trip. Personal group-level exposures of $PM_{2.5}$, total and size-selective particle counts, BC, temperature, and relative humidity will be collected on two portable carts, which will follow the participant group throughout their trip and will be placed in a central location inside the residence at other times during each monitoring week, beginning 24-hr prior to the first bus trip and ending 24-hr after the trip. [When the participants are in the health assessment room, the carts will be placed in this room.] Since the participants on each trip will live in the same residence facility and since the monitoring carts will follow them throughout the day, these group-level personal measurements will serve as a cost-effective yet accurate measure of each participant's pollutant exposures. Personal measurements will be supplemented by continuous measurements of O_3, CO, NO, and NO_x, which will be collected inside the bus when in operation, and by ambient particulate and gaseous monitoring data from the Countway SAM and State SAM sites, which will be used to provide a measure of the regional particulate exposures for the study cohort, particularly for periods more than one day prior to the bus trips. Ambient air pollution data will include measurements of $PM_{2.5}$, PM_{10}, sulfate (SO_4^{2-}), BC, EC, organic carbon (OC), O_3, SO_2, NO_X, NO_2, CO concentrations. Personal and ambient air pollution monitoring methods are described in detail in the Particle Technology and Monitoring Core (Core B).

4.5. Data Management: Blood samples will be tracked and stored at the Channing Laboratory using a bioinformatics management system (ORAGEN). This system was written in Visual Basic, using the MSJET database engine to manage relational data items. The ORACLE database engine (with which ORAGEN is fully compatible) will be used in place of the MSJET engine to ensure the highest attainable data quality standards. Subject data will be identified using only a 12-digit ID number created by ORAGEN. Only the Principal Investigator and the data programmer will be able to track the name of a subject, which will be linked to the ID number through ORAGEN. Write-access to the data will be restricted by password to the Principal Investigator and the data programmer. Other study accounts will have read-only access. All health and exposure assessment data are validated, checked and archived. All manuscripts with their associated programs will be archived centrally.

4.6. Data Analysis: The broad focus of this study is to examine associations between particulate air pollution and cardiovascular health, with particular emphasis on the effects of traffic related particles and gases. We will use a crossover study design to evaluate ambient and personal particle effects on inflammatory (pulmonary and systemic), vascular, and autonomic outcomes that are known to be associated with coronary artery disease and cardiac morbidity and mortality. Data will be examined alone initially and subsequently together with our earlier St. Louis bus study. In addition to our primary hypotheses, we will also examine (1) whether the risk of autonomic dysfunction (reduced HRV) with increased exposure to mobile source-related particle pollution is modified by disease status and (2) the association between mobile source-related exposures and both oxygen saturation and blood pressure, which will be considered secondary potentially interrelated intermediate outcomes involved in the pathways leading to cardiac morbidity in vulnerable populations. Outcomes of interest will include HRV, eNO, and measures of systemic inflammation in the blood (CRP, ICAM, VCAM, IL-6, among others). The approach and methods used to test our hypotheses are presented in the Biostatistics Core in Section 4.2.2.

4.6.1. Power Calculations: Our power calculations for repeated measures designs use variance formulas for linear regression and the fact that the mixed model can be expressed as a series of such regressions[69]. Because scientific interest focuses on both the overall effect of pollution in the proposed study as well as effect modification between the more frail individuals selected in this study and the relatively healthy individuals recruited in the St. Louis study, we calculated power for both the main effect of pollution in the proposed cross-over study, as well as differences in the dose-response between the two studies.

Table 4. Power of Boston Bus Study for Published Main Effects of Pollution Exposure and Minimal Detectable Differences (MDD) for Effect Modification

Outcome	**Power (Main Effect)**		**$PM_{2.5}$ * Cohort MDD**
	$PM_{2.5}$	**BC**	
HRV: r-MSSD	100%	100%	-6.1% vs. -9.3% per 14.3 ug/m^3
Systemic Inflammation (CRP)	97%	99%	+147% vs. +483% per 100 ug/m^3
Pulmonary Inflammation (Exhaled NO)	99%	99%	+20% vs. +30% per 10 ug/m^3

We first estimated the power of the proposed Boston bus study alone to detect effects of $PM_{2.5}$ and BC on the primary outcomes HRV, CRP, and eNO. For inputs, we used estimates of the within- and between-person variability for each outcome, as observed in similar studies conducted by our group. Because the proposed crossover design is slightly different from that used in existing studies, we calculated assumed effect sizes in the following way. For concreteness, consider eNO. The St. Louis analysis estimates a dose-response relationship with BC post-trip of a 20% increase per 500 ng/m^3 (Table 2). As shown on Figure 1, BC exposures for subjects on the two buses will be approximately 2,500 ng/m^3. Thus, a reasonable difference in eNO for subjects undergoing the two bus exposure scenarios is $\exp((2{,}500/500)*\log(1.20)) = 2.0$, or a 100% increase in eNO. We calculated analogous effect sizes for HRV and CRP. Based on results from our Summer 1999 panel study of older adults, we assumed a -6.1% change in r-MSSD per 14.3 increase in $PM_{2.5}$ and -10.1% change in r-MSSD per IQR increase in BC. For CRP, we assumed a 147% increase in CRP per 100 ug/m^3 increase in PM, as reported in Seaton *et al*. (1999)[31], and an increase of 0.05 mg/dl per 500 ng/m^3 increase in BC from preliminary analyses of the NAS study (as described in Project 1). As shown in Table 4, we will have sufficient power (>95%) for these assumed effect sizes to detect expected main effects of $PM_{2.5}$ and BC for all outcomes based solely on the proposed sample size of 72 person-trips.

Because less is known about effect modification, we calculated minimal detectable interactions between the St. Louis and Boston studies, at 80% power. Due to the different designs in the two studies, we calculated these minimal detectable differences (MDD) in terms of dose-response slopes. Assuming that the St. Louis group (n=44) exhibits the effect sizes given above, we estimated the minimal detectable $PM_{2.5}$ effect in the Boston study given the proposed sample size of 36 (Table 4). Estimated minimal detectable differences also apply to effect modification by clinical factors, such as obesity, hypertension, or metabolic syndrome, as long as data from both studies are included in the analyses, since the prevalence of these factors roughly match the Boston/St. Louis split in the total sample. Results suggest that we will have sufficient power to detect effect modification.

4.8. Timeline: The study will be conducted in Years 3 through 5 of the Center. Beginning in Year 3, study participants will be recruited and screened, particle removal technology for the buses will be prepared and validated, and other field preparations will take place. Field trips and health and exposure monitoring will occur in the spring and fall of Year 4. Statistical analyses will begin upon finalization of data sets, which will be based on appropriate quality assurance and control steps and is expected to begin at the start of Year 5 of the Center.

5. EXPECTED BENEFITS

Results from this study will provide key information about the impact of air pollution from motor vehicles on the cardiovascular health of older adults with underlying cardiopulmonary diseases and about the relative importance of particulate and gaseous exposures on these impacts. By using bus trips and personal group level exposure measures, the effects of motor vehicle related pollution on cardiovascular health can be examined specifically, absent from concerns over correlations with pollutants from other major particulate sources. As a follow-up to our St. Louis study, the proposed study will provide an important link to our previous Center research and will allow us to examine whether the risks posed by motor vehicle particulate and gaseous pollution differ based on the underlying disease or health status of the individual. Furthermore, the proposed study complements other Center studies, as it is based on similar health outcomes and pollutant measures, has a similar design to our controlled human and animal studies, and studies “free living” individuals exposed to non-concentrated motor vehicle pollution.

6. GENERAL PROJECT INFORMATION

The study will be directed by Dr. Helen Suh, the Principal Investigator of the study, together with the study co-investigator Dr. Gold. The study will rely on the expertise of the Exposure and Biostatistics Core, who will guide the exposure measurements and statistical analyses, respectively. Drs. Suh, Gold, Speizer, Schwartz, Stone, Coull and Koutrakis together with the Project Coordinator and other key study personnel will meet bi-monthly to discuss study progress, study-related issues or problems, and coordination with other Center projects. Field trips will be conducted using two identical buses; the bus with modified exposures owned by Harvard and the second, unmodified bus rented from the manufacturer.

7. REFERENCES

1. Katsouyanni K, Touloumi G, Spix C, Schwartz J, Balducci F, Medina S, Rossi G, Wojtyniak D, Sunyer J, Bacharova L, Schouten JP, Pontak A, HR Anderson. Short term effects of ambient sulphur dioxide and particulate mater on mortality in 12 European cities: results from time series data from the APHEA project. *Brit Med J* 1997; 314: 1658–1663.
2. Borja-Aburto VH, Loomis DP, Shrinkant IB, Shy CM, Mascon-Paceco RA. Ozone, Suspended Particles, and Daily Mortality in Mexico City. Amer J Epid 1997; 145(3): 258-68.
3. Pope CA, Dockery DW, Schwartz J. Review of Epidemiologic Evidence of Health Effects of Particulate Air Pollution. *Inhal Toxicol* 1995; 7: 1–18.
4. Air Quality Criteria for Particulate Matter. Second External Review Draft. US EPA, Office of Research and Development, Research Triangle Park, NC, March 2001.
5. Samet J, Zeger S, Kelsall J, Xu J, Kalkstein L. Does weather confound or modify the association of particulate air pollution with mortality? An analysis of the Philadelphia data, 1973-1980. *Environ Res* 1998; 77: 9-19.
6. Zanobetti A, Schwartz J, Dockery DW. Airborne particles are a risk factor for hospital admissions for heart and lung disease. *Environ Health Perspect* 2000; 108: 1071-1077.
7. Samet JM, Zegar SL, Dominici F, Curriero F, Coursac I, Dockery DW, Schwartz J, Zanobetti A. The National Morbidity, Mortality, and Air Pollution Study Part II: Morbidity, Mortality, and Air Pollution in the United States. *Health Effects Institute*, 2000; 94: 1-84.
8. Schwartz J. Assessing Confounding, Effect Modification, and Thresholds in the Association between Ambient Particles and Daily Deaths. *Environ Health Perspect*, 2000: 108:563-568.
9. Schwartz J. Particulate air pollution and daily mortality in Cincinnati, OH. *Environ Health Perspect* 1994; 102: 186-9.
10. Schwartz J, Morris R. Air pollution and hospital admissions for cardiovascular disease in Detroit, Michigan. *Am J Epid* 1995; 142: 22-35.
11. Schwartz J. Air pollution and hospital admissions for heart disease in eight US counties. Epid 1999; 10: 17-22.
12. Burnett RT, Dales R, Krewski D, Vincent R, Dann T, Brook JF. Associations between ambient particulate sulfate and admissions to Ontario hospitals for cardiac and respiratory diseases. *Am J Epid* 1995; 142: 15-22.
13. Schwartz J, Dockery D, Neas L. Is daily mortality associated specifically with fine particles? *JAWMA* 1996; 46: 927-939.
14. Bagley ST, Baumgard KJ, Gratz LD, Johnson H. Characterization of fuel and after treatment device effects on diesel emissions. *Res Resp Health Eff Inst* 1996; 1-75.
15. Laden F, Neas LM, Dockery DW, Schwartz J. Association of fine particulate matter from different sources with daily mortality in six US cities. *Environ Health Perspect* 2000; 108: 941-947.
16. Hoek G, Brunekreef B, Goldbohm S, Fischer, P, van den Brandt PA. Associations between mortality and indicators of traffic-related air pollution in the Netherlands: a cohort study. *Lancet* 2002; 360: 1203-1209.
17. Pekkanen J, Brunner EJ, Anderson HR, Tiittanen P, Atkinson RW. Daily concentrations of air pollution and plasma fibrinogen in London. *Occup Environ Med* 2000; 57(12): 818-822.
18. Janssen NA, Schwartz J, Zanobetti A, Suh HH. Air conditioning and source-specific particles as modifiers of the effect of PM10 on hospital admissions for heart and lung disease. *Environ Health Perspect* 2002; 110: 43-9.

19. Godleski JJ, Verrier RL, Koutrakis P, Catalano P, Coull B, Reinisch U, Lovett EG, Lawrence J, Murthy GG, Wolfson JM, Clarke RW, Nearing BD, Killingsworth C. Mechanisms of morbidity and mortality from exposure to ambient air particles. *Res Rep Health Eff Inst*. 2000; Report number 91: pp 5-88; peer discussion pp 89-103.
20. Electrophysiology. Special Report: Heart rate variability standards of measurement, physiological interpretation, and clinical use. *Circulation* 1996; 93: 1043-1065.
21. Kleiger RE, Miller JP, Bigger JT, Jr., Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am J Cardiol* 1987; 59: 256-62.
22. Sapin PM, Koch G, Blauwet MB, McCarthy JJ, Hinds SW, Gettes LS. Identification of false positive exercise tests with use of electrocardiographic criteria: a possible role for atrial repolarization waves. *J Am Coll Cardiol* 1991; 18: 127-35.
23. Malfatto G, Beria G, Sala S, Bonazzi O, Schwartz PJ. Quantitative analysis of T wave abnormalities and their prognostic implications in the idiopathic long QT syndrome. *J Am Coll Cardiol* 1994; 23: 296-301.
24. Nearing BD, Huang AH, Verrier RL. Dynamic tracking of cardiac vulnerability by complex demodulation of the T wave. *Science* 1991; 252: 437-40.
25. Bigger JT, Jr., Fleiss JL, Steinman RC, Rolnitzky LM, Kleiger RE, Rottman JN. Frequency domain measures of heart period variability and mortality after myocardial infarction. *Circulation* 1992; 85: 164-71.
26. Bigger JT, Jr., Fleiss JL, Rolnitzky LM, Steinman RC. Frequency domain measures of heart period variability to assess risk late after myocardial infarction. *J Am Coll Cardiol* 1993; 21: 729-36.
27. Gold DR, Litonjua A, Schwartz J, *et al*. Ambient pollution and heart rate variability. *Circulation* 2000; 101: 1267-73.
28. Magari SR, Hauser R, Schwartz J, Williams PL, Smith TJ, Christiani DC. Association of heart rate variability with occupational and environmental exposure to particulate air pollution. *Circulation* 2001; 104: 986-91.
29. Schwartz J, Suh H, Verrier M *et al*. Fine combustion particles and heart rate variability in an elderly panel. *Epid* 2001; 12: S64.
30. Seaton A, MacNee W, Donaldson K, Godden D. Particulate air pollution and acute health effects. *Lancet* 1995; 345:176-8.
31. Seaton A, Soutar A, Crawford V, Elton R, McNerlan S, Cherrie J, Watt M, Agius R, Stout R. Particulate air pollution and the blood. *Thorax* 1999; 54(11): 1027-1032.
32. Kharitonov SA, Barnes PJ. Nitric oxide in exhaled air is a new marker of airway inflammation. *Monaldi Arch Chest Dis* 1996; 51: 533-7.
33. Chatkin JM, Ansarin K, Silkoff PE, *et al*. Exhaled nitric oxide as a noninvasive assessment of chronic cough. *Am J Respir Crit Care Med* 1999; 159: 1810-3.
34. Clini E, Bianchi L, Ambrosino N. Exhaled nitric oxide in COPD patients. *Monaldi Arch Chest Dis* 2001; 56:169-70.
35. Kharitonov SA, Yates D, Springall DR, *et al*. Exhaled nitric oxide is increased in asthma. *Chest* 1995; 107: 156S-157S.
36. Barnes PJ, Liew FY. Nitric oxide and asthmatic inflammation. *Immunol Today* 1995; 16: 128-30.

37. Van Amsterdam JG, Verlaan BP, Van Loveren H, *et al*. Air pollution is associated with increased level of exhaled nitric oxide in nonsmoking healthy subjects. *Arch Environ Health* 1999; 54: 331-5.
38. Steerenberg PA, Snelder JB, Fischer PH, Vos JG, van Loveren H, van Amsterdam JG. Increased exhaled nitric oxide on days with high outdoor air pollution is of endogenous origin. *Eur Respir J* 1999; 13: 334-7.
39. Koenig JQ, Jansen K, Mar TF, *et al*. Measurement of offline exhaled nitric oxide in a study of community exposure to air pollution. *Eviron Health Perspect* 2003; 111: 1625.
40. Adamkiewicz G, Ebelt S, Syring M, Slater J, Speizer E, Schwartz J, Suh H, Gold DR. Association between air pollution exposure and exhaled nitric oxide in an elderly panel. *Thorax* 2003; 59: 204-209.
41. Albert MA, Ridker PM. The role of C-reactive protein in cardiovascular disease risk. *Curr Cardiol Rep* 1999; 1: 99-104.
42. Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14,719 initially healthy American women. *Circulation* 2003; 107: 391-7.
43. Pradhan AD, Rifai N, Ridker PM. Soluble intercellular adhesion molecule-1, soluble vascular adhesion molecule-1, and the development of symptomatic peripheral arterial disease in men. *Circulation* 2002; 106: 820-5.
44. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 2001; 286: 327-34.
45. Schwartz J. Air pollution and blood markers of cardiovascular risk. *Environ Health Perspect* 2001; 109 Suppl 3: 405-9.
46. Peters A, Doring A, Wichmann E, Koenig W. Increased plasma viscosity during an air pollution episode: a link to mortality? *Lancet* 1997; 349: 1582-1587.
47. Ghio AJ, Kim C, Devlin RB. Concentrated ambient air particles induce mild pulmonary inflammation in healthy human volunteers. *Am J Resp Crit Care Med* 2000; 162: 981-988.
48. Gardner SY and Costa DL. Particle-induced elevations in white blood cell count and plasma fibrinogen levels in rats. *Am J Resp Crit Care Med* 1998; 157: A152.
49. Zmirou D, Schwartz J, Saez M, Zanobetti A, Wojtymiak B, Touloumi G, Spix C, Ponce de Leon A, LeMoullec Y, Bacharova L, Schouten J, Ponka A, Katsouyanni K. Time series analysis of air pollution and cause specific mortality: a quantitative summary in Europe (APHEA study). *Epid* 1998; 9(5): 495-503.
50. Salvi S, Blomberg A, Rudell B, Kelly F, Sandstrom T, Holgate ST, Frew A. Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers. *Am J Respir Crit Care Med*. Mar 1999; 159(3): 702-709.
51. Linn WS, Gong H, Jr., Clark KW, Anderson KR. Day-to-day particulate exposures and health changes in Los Angeles area residents with severe lung disease. *JAWMA* 1999; 49:108-15.
52. Ibald-Mulli A, Stieber J, Wichmann HE, Koenig W, Peters A. Effects of air pollution on blood pressure: a population-based approach. *Am J Publ Health* 2001; 91:571-7.
53. Zanobetti A, Canner MJ, Stone PH, Schwartz J, Sher D, Eagan-Bengston E, Gates KA Batley LH, Suh HH Gold DR. Ambient Pollution and Blood Pressure in cardiac rehabilitiation patients. *Circulation* in press 2004.

54. Muller JE, Abela GS, Nesto RW, Tofler GH. Triggers, acute risk factors and vulnerable plaques: the lexicon of a new frontier. *J Am Coll Cardiol* 1994; 23:809-13.
55. Takase B, Uehata A, Akima T, *et al*. Endothelium-dependent flow-mediated vasodilation in coronary and brachial arteries in suspected coronary artery disease. *Am J Cardiol* 1998; 82:1535-9, A7-8.
56. Brook RD, Brook JR, Urch B, Vincent R, Rajagopalan S, Silverman F. Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. *Circulation* 2002; 105:1534-6.
57. Zmirou D, Schwartz J, Saez M, Zanobetti A, Wojtymiak B, Touloumi G, Spix C, Ponce de Leon A, LeMoullec Y, Bacharova L, Schouten J, Ponka A, Katsouyanni K. Time series analysis of air pollution and cause specific mortality: a quantitative summary in Europe (APHEA study). *Epid* 1998; 9(5): 495-503.
58. Van Amsterdam JG, Verlaan BP, Van Loveren H, *et al*. Air pollution is associated with increased level of exhaled nitric oxide in nonsmoking healthy subjects. *Arch Environ Health* 1999; 54:331-5.
62. Nitta H, Sato T, Nakai S, Maeda K, Aoki S, Ono M. Respiratory health associated with exposure to automobile exhaust. I: results of cross-sectional studies in 1979, 1982, and 1983. *Arch Environ Health* 1993; 48: 53-8.
63. Brunekreef B, Janssen NAH, DeHartog J, Hassema H, Knafe M, Van Vliet P. Air pollution from truck traffic and lung function in children living near motorways. *Epid* 1997; 8:298-303.
64. Kinney PL, Aggarwal M, Northridge ME, Janssen NAH, Shepard P. Airborne concentrations of $PM_{2.5}$ and diesel exhaust particles on Harlem sidewalks: a community-based pilot study. *Environ Health Perspect* 2000; 108(3):213-218.
65. Koutrakis P, Suh H, Sarnat J, Brown K, Coull B, Schwartz J. Characterization of particulate and gaseous exposures of sensitive sub-populations living in eastern US metropolitan areas – *Health Effects Institute, Final Investigators Report*. 2004.
66. Chang LT, Koutrakis P, Catalano P, Suh HH. Hourly personal exposures to fine particles and gaseous pollutants-results from Baltimore, MD. *JAWMA* 2000; 50:1223-35.
67. Recommendations for standardized procedures for the on-line and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-1999. Official statement of the American Thoracic Society, adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 1999; 160: 2104-17.
68. Massaro AF, Mehta S, Lilly CM, Kobzik L, Relly JJ, Drazen JM. Elevated nitric oxide concentrations in isolated lower airway gas of asthmatic subjects. *Am J Respir Crit Care Med* 1996: 153: 1510-4.
69. Fitzmaurice G, Laird N, and Ware J. Applied Longitudinal Analysis. Chapter 15. Wiley Europe Publishers, 2004.