ABSTRACT

Title of Dissertation: ASSOCIATIONS BETWEEN AMBIENT PARTICULATE MATTER EXPOSURES, STROKE, AND MARKERS OF CARDIOVASCULAR INFLAMMATION

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Dissertation directed by: Professor Robin Puett, Maryland Institute of Applied Environmental Health

Stroke is a leading cause of morbidity and mortality in the United States with 795,000 people experiencing a new or recurrent stroke every year. Identifying modifiable risk factors for stroke should therefore be considered a research priority. While associations between ambient exposure to air pollution and other cardiovascular diseases are well established in the literature, the evidence linking particulate matter (PM) air pollution exposures to the risk of ischemic or hemorrhagic stroke remains equivocal. Furthermore, the exact pathophysiologic mechanisms by which exposure to PM may lead to cerebrovascular events are not yet fully understood. Hypothesized pathways include the mediation of effects through a combination of inflammatory responses, autonomic dysregulation, and/or vascular endothelial disturbances. This dissertation addresses existing gaps in the literature in three separate studies. Two time-stratified case-crossover studies examined the association between short-term PM exposures and stroke risk, one in the Health Professionals Follow-up Study

(HPFS) and the other among a large database of Maryland stroke hospitalizations. Conditional logistic regression models were used to examine associations by stroke subtype, population subgroups, and clinically-relevant variables. Our third study took place within the Nurses' Health Study cohort. Multivariable linear regression models were used to examine the associations between PM and residential distance to road exposures and four inflammatory biomarkers (CRP, IL-6, fibrinogen, and ICAM-1). We found positive significant associations between PM_{10} and ischemic stroke events in the HPFS cohort, and associations were elevated for nonsmokers, aspirin nonusers, and those without a history of high cholesterol. Concentrations were elevated for both CRP and IL-6 among participants who lived close to a major roadway, but no significant results were found by estimated PM exposure. This work provides additional evidence that PM exposure is associated with ischemic stroke and adds to the current literature that those not currently taking aspirin and those without a history of high cholesterol may be at elevated risk. Although the direct role of inflammatory processes requires more investigation, this work does provide additional evidence that proximity to traffic may influence cardiovascular-related inflammation.

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ASSOCIATIONS BETWEEN AMBIENT PARTICULATE MATTER EXPOSURES, STROKE, AND MARKERS OF CARDIOVASCULAR INFLAMMATION

by

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Dedication

This work is dedicated to my wife, Victoria and daughter, Sophie. Victoria, thank you for your dedication, sacrifices, encouragement, and unwavering support throughout my entire graduate program, especially on those long nights or weekends at the office. Your patience and love knows no bounds. Sophie, thank you for the extra inspiration and motivation to finish this work. I love you both.

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List of Abbreviations

AQS	Air Quality System
BMI	Body-mass Index
CDC	Centers for Disease Control
CI	Confidence Interval
CRP	C-reactive Protein
EPA	Environmental Protection Agency
ESCAPE	European Study of Cohorts of Air Pollution Effects
HPFS	Health Professionals Follow-up Study
Hs-CRP	High-sensitivity C-reactive Protein
ICAM-1	Intercellular Adhesion Molecule
ICD	International Classification of Disease
IL-6	Interleukin 6
IQR	Interquartile Range
km	Kilometer
m	Meter
MD	Maryland
MESA	Multi-Ethnic Study of Atherosclerosis
MI	Myocardial Infarction
NAAQS	National Ambient Air Quality Standards
NHANES	National Health and Nutrition Examination Survey
NHS	Nurses' Health Study
NO_2	Nitrogen Dioxide
NO_x	Nitrogen Oxides
OR	Odds Ratio
PM	Particulate Matter
PM_{10}	Particulate Matter (Less than 10 microns in diameter)
$PM_{2.5}$	Particulate Matter (Less than 2.5 microns in diameter)
$PM_{2.5-10}$	Particulate Matter (Between 2.5 and 10 microns in diameter)
RR	Risk Ratio
SD	Standard Deviation
TF	Tissue Factor
TRAP	Traffic-related Air Pollution
TSP	Total Suspended Particulate
µg	Micrograms

Chapter 1: Introduction

Dissertation Overview

Associations between ambient exposure to air pollution and cardiovascular mortality and morbidity are well established in the literature. In fact, a recent scientific statement from the American Heart Association based on a comprehensive review of current evidence concluded exposure to fine particles (diameter <2.5 μm, $PM_{2.5}$) represents a causal and modifiable risk factor for cardiovascular disease (Brook et al. 2010).

While studies focused on general cardiovascular outcomes date back decades, only recently have studies demonstrated associations between ambient pollution and increased stroke risk. A major focus of this research has been whether high transient levels of air pollution lead to sudden stroke onset. To date, the evidence linking short-term changes in PM to the risk of cerebrovascular events remains equivocal, with some (Dominici et al. 2006; Wellenius GA et al. 2012; Zanobetti and Schwartz 2009) but not all (Anderson et al. 2001; O'Donnell et al. 2011) studies finding evidence of increased risk. As many of the existing studies of PM and stroke have used administrative databases that may be subject to misclassification of outcome data, a recent review (Ljungman and Mittleman 2014) has called for additional studies with validated diagnostic criteria and studies which examine risk by stroke subtype (ischemic, hemorrhagic). Additionally, few studies have been able to examine potential effect modification by clinically-relevant individual-level variables or have

been conducted among study populations of sufficient size to examine differences in risk by population subgroups.

The exact pathophysiologic mechanisms by which exposure to air pollution leads to stroke are not yet fully understood. Hypothesized pathways for air pollution-related stroke include increased systemic inflammation and oxidative stress leading to thrombosis and vascular endothelial dysfunction (Mills et al. 2007). Though existing literature is sparse, there is some evidence that air pollutants could induce an acute systemic inflammatory response with an increased number of circulating fibrinogen, C-reactive protein and proinflammatory cytokines (Pope 2001; van Eeden et al. 2001), which could result in increased blood coagulation and plaque destabilization and rupture (Mills et al. 2007). Additional epidemiologic studies linking ambient particulate matter exposure and subsequent markers of systemic and cardiovascular inflammation are needed.

To address these research gaps the following aims are proposed:

Aim #1) Examine the association between ambient particulate matter exposures and short-term stroke risk in the Health Professionals Follow-up Study

a) Examine risk of stroke from exposure to both PM_{10} and $PM_{2.5}$
b) Examine associations for each stroke subtype (ischemic, hemorrhagic)
c) Examine effect modification by BMI, smoking status, age categories, hyperlipidemia, current aspirin use, and hypertension status

Aim #2) Examine the association between acute changes in particulate matter concentrations and stroke hospital admissions in Maryland

a) Examine associations for each stroke subtype (ischemic, hemorrhagic)

b) Examine effects by season and among population subgroups (age, gender, race/ethnicity)

Aim #3) Determine the association between ambient particulate matter exposures and systemic and cardiovascular inflammatory markers, including C-reactive protein (CRP), fibrinogen, soluble intercellular adhesion molecule type-1 (sICAM-1), and interleukin-6 (IL-6) among individuals in the Nurses' Health Study

a) Examine the association by particulate matter fraction ($PM_{2.5}$, PM_{10}, $PM_{2.5-10}$)
b) Examine association with various cumulative exposure averaging times (1, 3, 12 months)
c) Examine potential effect modification by smoking status

Background and Rationale

Significance

Stroke is a leading cause of morbidity and mortality in the United States with 795,000 people experiencing a new or recurrent stroke every year (Mozaffarian et al. 2015). Of these strokes, approximately 130,000 result in mortality, making stroke the fifth leading cause of death in the US (CDC 2016b; Mozaffarian et al. 2015). Case fatality rates vary by stroke subtype. Ischemic strokes, which occur as a result of an obstruction within a blood vessel, are the most frequently occurring stroke subtype and comprise 87% of all stroke cases with 8 to 12% resulting in death within 30 days. Though they only comprise 10% of all stroke cases, hemorrhagic strokes, which occur when a weakened blood vessel in the brain ruptures, are significantly more

deadly (37% result in death within 30 days) (Mozaffarian et al. 2015; Rosamond et al. 1999). Age is the most significant risk factor for stroke, with risk approximately doubling for each decade of life after age 55 (AHA 2015b). More women have strokes than men and higher rates of stroke are also seen among African-Americans. Modifiable risk factors for stroke include high blood pressure, smoking, diabetes, atrial fibrillation, high blood cholesterol, poor diet, and obesity. Depending on the severity and region of the brain affected, stroke can cause significant disability, including paralysis, speech impairment, coma, or loss of memory and reasoning ability (AHA 2015a). In 2011, the direct and indirect cost of stroke was $33.6 billion, and between 2012 and 2030 the total direct medical stroke-related costs are expected to triple (Mozaffarian et al. 2015; Ovbiagele et al. 2013).

Particulate matter (PM), or particle pollution, is the term used for a mixture of solid particles and liquid droplets suspended in the air. Particles vary in their chemical and physical compositions and originate from a variety of sources such as motor vehicle combustion, brake & tire wear, power plants, wood or agricultural burning, forest fires, or can be formed in the atmosphere from chemical reactions to gaseous emissions. Particles are generally classified by size, or more specifically, their aerodynamic diameter which correlates to the size of a sphere with the same aerodynamic characteristics. It is useful to classify particles by their aerodynamic size because aerodynamic size can dictate: (a) the transport of particles in the air; (b) their deposition within the respiratory system and (c) the association with the particle's source and chemical composition (WHO 2003). Though airborne particles can range in size from a few nanometers to tens of micrometers, the most commonly referred to

particulate size fractions in human health research are coarse particles ($PM_{2.5-10}$; between 2.5um and 10um in aerodynamic diameter) and fine particles ($PM_{2.5}$; less than 2.5um in aerodynamic diameter) [Note - the designation PM_{10} refers to both coarse and fine particulates; all particles <10um]. Coarse particles are primarily formed by abrasive mechanical processes that break-up larger particles. These particles can include wind-blown dust from agricultural processes or uncovered soil or abrasively-derived traffic-related particles (brake and tire wear). Coarse particles from environmental sources include those from the evaporation of sea spray as well as pollen grains and mold spores. Coarse particles are generally only suspended in the air from minutes to hours and generally travel short distances (<10km) (US EPA 2003). Fine particulates differ from coarse particles both in their origin and chemistry. These particles are formed by combustion, high-temperature processes, and atmospheric reactions. They are composed of a variety of carbon and organic compounds, sulfates, nitrates, and metals (Pb, Cd, V, Ni, Cu, Zn, Mn, and Fe). Chemical and physical compositions vary by location, time of year, and weather. In contrast to coarse particles, the lifetime for fine particles in the atmosphere can be days to weeks and they can travel thousands of kilometers (US EPA 2003).

In the early to mid-twentieth century, the risks of air pollution on health became readily apparent after several widely publicized acute episodes of increased morbidity and mortality followed exceptionally high spikes in pollution levels as a result of temperature inversions (Jun 2009). The most dramatic and infamous of these episodes were in the Meuse Valley in Belgium in 1930, Donora, Pennsylvania in 1948, and London in 1952 (Firket 1936; Health 1954; Wexler and Schrenk 1949).

Each of these episodes demonstrated that severe air pollution can have dramatic and immediate impact on population health. During the London Fog episode, for example, approximately 4,000 to 12,000 excess deaths occurred, the greatest number of which were related to cardiovascular disease (Bell et al. 2004a; Health 1954). Additionally, a 10-year follow up of the exposed Donora population showed increased cardiorespiratory morbidity and mortality years afterward among those who complained of severe acute illness in 1948 (Ciocco and Thompson 1961). The significant health outcomes from these episodes likely played a large role in prompting many governments to initiate research on air pollution and health and enact legislation aimed at reducing air pollution levels (Bell et al. 2004b).

The Clean Air Act (established 1970, last revised 1990) requires the Environmental Protection Agency (EPA) to set National Ambient Air Quality Standards (NAAQS) for pollutants considered harmful to public health and the environment. Starting in 1971, the US EPA established the first PM standard as Total Suspended Particulates (TSPs). However, in 1987, this standard was replaced with PM_{10} as a decision to regulate only particles that penetrated to the thoracic region of the respiratory tract (Greenbaum et al. 2001). In 1997, after reviewing scientific studies showing differential health effects of particulates based on aerodynamic diameter, the EPA established new standards for $PM_{2.5}$ (Greenbaum et al. 2001). Annual standards for $PM_{2.5}$ concentrations were set at 15 $\mu g/m^3$, and 24-h $PM_{2.5}$ standards were set at 65$\mu g/m^3$. The NAAQS were last revised in 2012 with a major change being that annual $PM_{2.5}$ standards were divided into a primary (12 $\mu g/m^3$) and secondary (15$\mu g/m^3$) standard (US EPA 2017c). Primary standards are meant to

provide public health protection to sensitive populations (asthmatics, children, elderly), while secondary standards provide public welfare protection (decreased visibility, damage to animals crops). Nonattainment areas are regions that do not meet the NAAQS standards. As of February 13th, 2017 there were 20 counties within nine states that were designated nonattainment areas by the EPA for $PM_{2.5}$, and an estimated 23 million people live within these areas (US EPA 2017a). A total of 31 counties in 10 states encompassing over 9 million people are designated as non-attainment areas based on the 1987 PM_{10} standard (US EPA 2017b).

Particulate Matter Exposures and Stroke

Recently, studies examining associations between ambient pollution and increased stroke risk have gained more attention. A major focus of this research has been whether high transient levels of air pollution may lead to sudden stroke onset. To date, the evidence linking short-term changes in $PM_{2.5}$ and PM_{10} to the risk of cerebrovascular events remains equivocal. A review of previous studies which have examined the association between short-term exposure to PM and total cerebrovascular events, ischemic stroke, and hemorrhagic stroke follows.

Total Cerebrovascular Events and PM

Several studies have examined short-term effects of total cerebrovascular events without distinguishing between the subset of ischemic, hemorrhagic, or other or unclassified stroke subtypes (Alessandrini et al. 2013; Anderson et al. 2001; Bell et al. 2008; Delfino et al. 2009a; Dominici et al. 2006; Halonen et al. 2009; Jalaludin et

al. 2006; Larrieu et al. 2007; Le Tertre et al. 2002; Lippmann et al. 2000; Nascimento et al. 2012; Wong et al. 1999; Wordley et al. 1997; Zheng et al. 2013). Small but significant positive associations have been noted in several studies. In the largest study, Dominici et al. (2006) evaluated the association with hospital admission for total cerebrovascular disease and $PM_{2.5}$ among 11.5 million Medicare beneficiaries residing in 204 US urban counties and found a statistically significant 0.8% (95% CI 0.3% to 1.3%) higher risk per 10μg/m^3 increase in $PM_{2.5}$ (Dominici et al. 2006). In their study of wildfire-related $PM_{2.5}$ exposures in California, Delfino (2009) also found significant increase in total daily hospital admissions for cerebrovascular disease (RR=1.019; 95% CI:1.004-1.035) (Delfino et al. 2009a). Additionally, two studies have found associations with cerebrovascular admissions and PM_{10}. Nascimento et al (2012) used stroke hospital admissions from Sao Paulo State, Brazil and found a positive significant association of stroke admission with PM_{10} (RR-1.137; 95% CI: 1.014-1.276). Similarly, Wordley et al. (1997) found a 2.1% increase in risk of cerebrovascular admissions (95% CI: 0.1-4.1) per 10μg/m^3 increase in PM_{10} in Birmingham, UK (Wordley et al. 1997). Other studies of cerebrovascular admissions and $PM_{2.5}$ and PM_{10} have found mixed or no effects. A time-series study of cerebrovascular hospitalizations in Rome found no association with $PM_{2.5}$ (RR=0.978; 95% CI: 0.956-1.000) or PM_{10} (RR=1.005; 95% CI: 0.988-1.021) (Alessandrini et al. 2013). In Taiwan, another study of cerebrovascular hospital admissions found no association with $PM_{2.5}$. They found a significant association with PM_{10}, but only for lag day 3 (% risk increase=2.64; 95% CI: 0.21-5.12) (Bell et al. 2008). Anderson et al. (2001) used administrative data from the United Kingdom

and found non-significant associations with both PM_{10} (% change RR= -3.3; 95% CI: -7.9 to 1.4) and $PM_{2.5}$ (% change = -1.6; 95% CI: -6.6 to 3.6) (Anderson et al. 2001) Halonen et al. (2009) also found no suggestion of effect for $PM_{2.5}$ (RR=0.997; 95% CI: 0.969-1.026) in a study of hospital admissions in Finland (Halonen et al. 2009). Two studies limited to admissions among the elderly (+65 years) found no associations between stroke admissions and either $PM_{2.5}$ or PM_{10} in Detroit Michigan (Lippmann et al. 2000) or Sydney, Australia (Jalaludin et al. 2006). Additional studies examining only PM_{10} exposures and cerebrovascular admissions have found no significant associations (Larrieu et al. 2007; Le Tertre et al. 2002; Wong et al. 1999; Zheng et al. 2013).

Ischemic Strokes and PM

A number of studies have specifically evaluated the association between PM_{10} and $PM_{2.5}$ and the risk of ischemic stroke (Andersen et al. 2010; Chan et al. 2006; Henrotin et al. 2007; Linn et al. 2000; Lisabeth et al. 2008; Low et al. 2006; Mechtouff et al. 2012; O'Donnell et al. 2011; Szyszkowicz 2008; Tsai et al. 2003; Vidale et al. 2010; Villeneuve et al. 2006; Wellenius GA et al. 2012; Wellenius et al. 2005). Some of these studies have found small positive significant associations between PM and ischemic stroke. In their study of elderly Medicare beneficiaries in nine US cities, Wellenius et al. (2005) reported a 1.03% (95% CI, 0.04% to 2.04%) increase in admissions on the same day per interquartile range increase in PM_{10} (Wellenius et al. 2005). In New York City, Low et al. (2006) reported a statistically significant increase in ischemic stroke hospitalizations per 10 $\mu g/m^3$ increase in PM_{10}

(Low et al. 2006). In Taiwan, Tsai et al. (2003) found a 5.9% (95% CI: 4.3-7.4, lag 0-2 days) excess risk of hospitalization for ischemic stroke per 10 $\mu g/m^3$ increase in PM_{10} after excluding days with mean daily temperature <20°C (Tsai et al. 2003). A suggestion of effect was seen in a study by Chan et al. (2006) in another study in Taiwan. The authors found a 3.0% (95% CI: -0.8 to 6.6, lag 3) and 1.6% (95% CI: -0.8 to 3.9, lag 3) excess risk per 10 $\mu g/m^3$ increase in $PM_{2.5}$ and PM_{10}, respectively (Chan et al. 2006). Several studies deserve increased attention due to the specificity of stroke outcome definition. Wellenius et al. (2012) performed medical review of 1705 Boston area patients to confirm ischemic stroke events. They estimated the odds ratio of ischemic stroke to be 1.11 (95% CI: 1.03-1.20) (*P*=.006) per interquartile range increase in $PM_{2.5}$ levels (6.4 $\mu g/m^3$) (Wellenius GA et al. 2012). They did not find differences in risk by history of diabetes, atrial fibrillation or hypertension, but found slightly higher associations among stroke cases greater than 75 years old (Wellenius GA et al. 2012). Using data from the French Dijon Stroke Registry, Henrotin et al. (2007) analyzed 1432 confirmed cases of ischemic stroke and found a positive but non-significant increase 0.9% (95% CI: -7.0 to 9.4) per 10 $\mu g/m^3$ increase in PM_{10} on the same day and a 1.1% (95% CI: -0.2 to 9.4) on the previous day (lag 1 day) (Henrotin et al. 2007). Lisabeth et al. (2008) used data on 2,350 confirmed cases of ischemic stroke and 1,158 cases of transient ischemic attack from the Brain Attack Surveillance in Corpus Christi Project (BASIC), a population-based stroke surveillance project designed to capture all strokes in Nueces County, Texas. The authors found borderline significant results on the same day (RR=1.03; 95% CI : 0.99–1.07) and previous day (RR=1.03; 95% CI : 1.00-1.07) for an interquartile range

increase in $PM_{2.5}$ (Lisabeth et al. 2008). O'Donnell et al used data from a stroke registry in Canada and found no association with PM among 9,202 ischemic stroke cases (O'Donnell et al. 2011). However, there was some evidence to suggest that associations differ among ischemic stroke etiologies. $PM_{2.5}$ was positively associated with strokes that were a result of small-vessel occlusions or large-artery atherosclerosis, but negatively associated with strokes due to cardioembolism. O'Donnell et al. also examined several effect modifiers of the PM stroke relationship. Risk was elevated among those with a history of diabetes compared to those without history of diabetes. They found no evidence that the associations differed by smoking status or history of hypertension. Mechtouff performed a medical review on 376 ischemic stroke cases and also found no association per SD increase in PM_{10} (OR=0.94; 95% CI: 0.80–1.10) or $PM_{2.5}$ (OR=0.97; 95% CI: 0.83–1.12) (Mechtouff et al. 2012). Additionally, two studies examining emergency department visits for acute ischemic stroke in Edmonton, Canada hospitals found no associations between ischemic stroke and $PM_{2.5}$ or PM_{10} (Szyszkowicz 2008; Villeneuve et al. 2006).

Hemorrhagic Strokes and PM

A subset of the studies examining effects of PM on ischemic stroke also looked at associations with hemorrhagic stroke. Only one study, Tsai et al. (2003), found a significant positive association between PM and hemorrhagic stroke. Among 4,359 hospital admissions, the authors of this study noted an OR of 1.54 (95% CI: 1.31-1.81) per IQR (66.3 $\mu g/m^3$) increase in PM_{10}, but only in days where the temperature was >20°C (Tsai et al. 2003). In contrast, Wellenius et al. (2005) found no evidence

of association between ambient PM_{10} levels and risk of hemorrhagic stroke among Medicare beneficiaries in nine U.S. cities (RR=0.997; 95% CI: 0.976-1.020) (Wellenius et al. 2005). Similarly, in their study of 2,329 emergency department visits for hemorrhagic stroke in Edmonton, Canada, Villeneuve et al. (2006) found no evidence of an association with either $PM_{2.5}$ (RR=0.984; 95% CI: 0.857-1.130) or PM_{10} (RR=1.006; 95% CI: 0.943-1.073) (Villeneuve et al. 2006). Henrotin et al. (2007) also found no evidence of an association between risk of hospitalization for hemorrhagic stroke and PM_{10} (RR=0.901; 95% CI: 0.731-1.111) among 220 strokes in a French stroke registry (Henrotin et al. 2007). Though Chan et al. found suggestions of increased risk for ischemic stroke in their study in Taipei, Taiwan, the same study found no evidence of association with hemorrhagic stroke hospitalizations with either $PM_{2.5}$ (RR=0.990; 95% CI: 0.954-1.028) or PM_{10} (RR=0.994; 95% CI: 0.974-1.014) (Chan et al. 2006).

A recent meta-analysis summarized the literature to date and found summary estimates of 1.4% (95% CI: 0.9-1.9) and 0.5% (95% CI: 0.3-0.7) higher risk for cerebrovascular mortality per $10ug/m^3$ of $PM_{2.5}$ and PM_{10}, respectively (Wang et al. 2014). Findings for ischemic stroke were suggestive, but the authors concluded that the weight of the published evidence does not yet show a statistically significant association (Wang et al. 2014). The authors also noted large heterogeneity between studies and suggested a number of reasons for this including; exposure and outcome misclassification, differing lag structures, varying methodologies in controlling for

meteorological variables, geographic variability in PM constituency, and variation in the demographic characteristics of study populations (Wang et al. 2014).

PM and Biomarkers of Cardiovascular Inflammation

Though current research suggests a link between cardiovascular outcomes and exposure to air pollutants, biological mechanisms for this association have not yet been fully explained. A clear understanding of these mechanisms is central to the development of better clinical treatment and prevention strategies. Many mechanisms and pathways have been proposed including: oxidative stress, changes in autonomic function, and pulmonary inflammation leading to thrombosis and atherosclerosis (Utell et al. 2002). Though exact mechanisms remain unclear, many hypothesize an important role of inflammatory processes. Toxic substances present in fine PM (e.g., black carbon, primary and secondary aerosols, metals) can cross epithelium of the airway following inhalation and induce the production of proinflammatory cytokines and reactive oxygen species (O'Toole et al. 2009; Uzoigwe et al. 2013). The effects produced by these air pollutants may then lead to hypertensive responses and changes in autonomic cardiac control (Du et al. 2016). Establishing epidemiologic associations between air pollutants and blood-borne markers of inflammation can provide needed evidence to these potential mechanistic pathways and should be considered a research priority.

The body of research exploring links between inflammatory markers and particulate exposure had been relatively limited before 2005. However, in the last decade there has been a growing interest in the field. While many of the studies have been limited

to fewer than 100 participants (usually panel studies and experimental studies), associations between inflammatory markers and air pollution exposures have also been examined among a few well-characterized large cohorts. Markers examined in these studies have included C-reactive protein, fibrinogen, homocysteine, IL-6, TNF-α, vascular cell adhesion molecule-1, intercellular adhesion molecule-1 (ICAM-1), soluble CD40 ligand, WBCs, and soluble adhesion molecules, but this review will focus on associations with CRP, fibrinogen, IL-6, and ICAM-1.

Biomarkers

C-reactive protein is a protein produced by the liver and is considered one of the best measures of the acute phase response to an infectious disease, tissue damage, or inflammation (Gabay and Kushner 1999; Pope 2001). CRP has been shown in multiple epidemiologic studies to predict incident myocardial infarction, stroke, and sudden cardiac death (Ridker 2003) and it may also have a direct role in the development of atherosclerosis (Libby 2002).

Fibrinogen is an essential blood-clotting glycoprotein. During the normal blood coagulation cascade, the protease thrombin converts soluble fibrinogen into insoluble fibrin strands which are cross-lined to form a blood clot. However, it has also been shown that there are distinct connections between inflammatory markers and the coagulation cascade, especially in regard to cardiovascular diseases (Demetz et al. 2012). A proinflammatory role for fibrinogen has been reported in stroke and

epidemiological associations have been found between fibrinogen and both coronary heart disease and myocardial infarction (Danesh et al. 1998; Davalos and Akassoglou 2012; Lind et al. 2001).

Interleukin 6 (IL-6) is a pro-inflammatory cytokine secreted by T cells and macrophages at the site of injury and is integral to the inflammatory response. Experimental studies have shown that human macrophages exposed to PM_{10} release numerous inflammatory cytokines, including IL-6 (Becker et al. 2005). Persistent inflammation in the lung may invoke a general systemic inflammatory response as pro-inflammatory cytokines diffuse into systemic circulation (Tamagawa et al. 2008).

Intracellular adhesion molecule-1 (ICAM-1) is a glycoprotein present on endothelial cells that possesses binding sites for immune-associated molecules including leukocytes and fibrinogen facilitating their migration to sites of inflammation (Witkowska and Borawska 2004). Elevated ICAM-1 levels have been shown to be associated with both cardiovascular risk factors such like hypertension and smoking (Blann et al. 1997; Chae et al. 2001; Rohde et al. 1999) as well as myocardial infarction and total acute coronary events (Haim et al. 2002; Ridker et al. 1998) .

Epidemiological Studies

Epidemiological studies linking associations between particulate matter exposures and CRP, fibrinogen, IL-6 and/or sICAM-1 follow:

Hajat et al (2015) examined 6,814 participants between 45 and 84 in the Multi-Ethnic Study of Atherosclerosis (MESA) between 2002 and 2012 (Hajat et al. 2015). The authors examined several day-long exposures and a cumulative 1 year exposure prior to blood draw. After controlling for confounders, a 5μg/m^3 increase in yearly $PM_{2.5}$ exposures was associated with 6% higher IL-6 (95% CI: 2 – 9%), but no associations for year-long exposure to soluble ICAM-1, fibrinogen or CRP. However, short-term $PM_{2.5}$ exposures on the day of blood draw were associated with CRP and fibrinogen. They also noted suggestion of effect modification with larger increases in IL-6 for older individuals, smokers, and participants with hypertension (Hajat et al. 2015). In another study of MESA participants, Diez-Roux et al examined whether CRP increased in response to changes in prior day, prior week, prior 30 days, or prior 60 day concentrations of $PM_{2.5}$ (Roux et al. 2006). In their study, the 30-day and 60-day mean exposures showed positive, but not statistically significant increases in CRP per 10μg/m^3 increase in $PM_{2.5}$ (30day OR= 1.05, 95% CI: 0.98-1.29; 60 day OR=1.12, 95% CI: 0.96-1.32) (Roux et al. 2006).

In one of the largest studies to date, Hampel et al (2015) used 21,558 high-sensitivity CRP (hs-CRP) measurements and 17,428 fibrinogen measurements from a total of nine European cohorts as part of the ESCAPE multi-center project (Hampel et al. 2015). For hs-CRP they found a non-significant association with both PM_{10} (1.2 % change; 95% CI: -3.8 to 6.4) and $PM_{2.5}$ (2.4% change; 95% CI:-7.5 to 13.4). They also found null results with the total pooled estimates for fibrinogen (PM_{10}=0.1% change; 95% CI:-1.4 to 1.7; $PM_{2.5}$=0.5 % change; 95% CI:-1.1 to 2.0). However, the

authors of this study were able to also look at associations by individual PM constituents. They found significant positive results between $PM_{2.5copper}$ and hs-CRP and between $PM_{2.5zinc}$ and fibrinogen (Hampel et al. 2015). Results from this study are important as they highlight that certain PM constituents may be the primary drivers of PM-related inflammatory changes.

Using data from 4,814 participants 45-75 years of age from three large German cities, Hoffman et al. examined changes in hs-CRP and fibrinogen in response to annual $PM_{2.5}$ exposures (Hoffmann et al. 2009). Per IQR increase in $PM_{2.5}$ (3.91 $\mu g/m^3$), significant increases were seen for men but not women with both hs-CRP (men: 23.9% increase; 95% CI: 4.1 to 47.4; women: 1.0% decrease; 95% CI: -16.5 to 17.3) and fibrinogen (men: 3.9% increase; 95% CI: 0.3 to 7.7; women: 1.5% increase; 95% CI: -1.9 to 5.1). The authors ran additional models and found that the inclusion of short-term exposures to $PM_{2.5}$, ozone, and temperature did not influence the results markedly. Another study in the same cohort did not find associations with hs-CRP and fibrinogen with year-long moving averages of PM_{10} (Viehmann et al. 2015).

In Boston, Zeka et al. (2006) studied 710 elderly members of the VA Normative Aging Study to examine changes in CRP and fibrinogen with acute changes in PM concentrations in the previous 48 h, 1-wk, and 4-wk (Zeka et al. 2006a). Though results were not statistically significant, they noted that associations were stronger for longer averaging periods of four weeks. Per 1 standard deviation (SD) increase in $PM_{2.5}$ for the 4-week period, they noted a 1.14 percent change in fibrinogen (95% CI:

-0.60 to 2.88) and a 4.36% change in CRP (95% CI: -3.25 to 11.96). They also observed associations were elevated among subjects older than 78 years and among the obese (Zeka et al. 2006a).

In another study examining association between CRP and $PM_{2.5}$, Ostro et al. included a total of 2,849 women ages 42-52 from 6 metropolitan centers across the U.S. (Ostro et al. 2014). The authors found overall that a 10μg/m^3 increase in 12-month average $PM_{2.5}$ levels (estimated by closest monitor within 20km) was associated with a 25.5% increase in CRP (95% CI: 10.2-42.9). They also noted that certain population groups were more susceptible to increases in CRP. Increases were higher among older individuals, diabetics, those with high BMI, and postmenopausal women. Results did not differ by smoking categories.

Several other large cross-sectional studies have been performed. In a cross-sectional study of 6,183 adults in Switzerland, Tsai (2012) found significant increases in IL-6 per 10μg/m^3 increase in PM_{10} (increase= 0.036 pg/mL; 95% CI: 0.015-0.057), but found no association with CRP levels (Tsai et al. 2012). Using NHANES III (1988-1994 only) data, Schwartz found that an IQR change in PM_{10} resulted in an odds ratio of 1.77 (95% CI: 1.26-2.49) for having a 90th percentile fibrinogen level (Schwartz 2001). In another large cross-sectional study of 3,659 individuals in Tel Aviv, Steinvil et al. found no associations between CRP and PM_{10} (measured at local monitors) either on the day of examination or 7 days prior (Steinvil et al. 2008).

Biological Plausibility

The exact pathophysiologic mechanisms by which exposure to air pollution leads to stroke and other cardiovascular outcomes are not yet fully understood. Hypothesized pathways for air pollution-related stroke include increased systemic inflammation and oxidative stress leading to procoagulant effects, autonomic irregularities, vascular endothelial dysfunction, and thrombosis which could enhance the likelihood of an ischemic event (Franchini and Mannucci 2007; Mills et al. 2007). Proposed mechanisms on the inflammatory pathway involve the secretion of adhesion molecules by damaged and inflamed pulmonary endothelial cells, which may result in increased binding and activation of leukocytes and platelets and the subsequent release of proinflammatory cytokines. Cytokines formed during tissue damage and inflammation lead to tissue factor (TF) induction which in turn stimulates coagulation following vascular injury (Choi et al. 2006). Complexes of TF on endothelial cell surfaces initiate the extrinsic blood coagulation pathway and play a key role in thrombin generation (Gilmour et al. 2005). Increased clotting may occur, as thrombin catalyzes many coagulation-related reactions, such as converting soluble fibrinogen into insoluble fibrin. Such mechanisms are supported by studies of animal models showing links between lung inflammation and extent of thrombosis following vascular injury (Nemmar et al. 2003) and the mediation of such effects through platelet-leukocyte activation (Nemmar et al. 2007). Another proposed mechanism is that particulate matter exposure can alter cardiac autonomic control of the heart leading to acute elevations in blood pressure and changes in atherosclerotic plaque stability (Franchini and Mannucci 2007; Wang et al. 2014). In one experimental study

of normotensive, non-smoking healthy adults, exposure to high levels of $PM_{2.5}$ rapidly increased diastolic blood pressure (Urch et al. 2005).

Objectives and Research Questions

Study 1 examined short-term ambient exposure to particulate matter and risk of stroke among participants of the Health Professionals Follow-up Study. Of major interest was the determination of risk for each stroke subtype (ischemic, hemorrhagic) and whether variables of clinical relevance modify the association between PM and stroke. Study 2 examined the association between short-term area-level fine particulate matter exposure and stroke hospitalization in Maryland. This study is of sufficient size that effect modifiers (season, age, gender, race/ethnicity) could be examined within both ischemic and hemorrhagic stroke hospitalizations. In both studies 1 and 2, we were also interested in whether associations varied depending on the timing of exposure prior to the stroke event, thus, multiple lag periods were examined in separate models. The objective of study 3 was to examine the relationship between particulate matter exposures and systemic and cardiovascular inflammatory markers, including C-reactive protein (CRP), fibrinogen, intercellular adhesion molecule type-1 (ICAM-1), and interleukin-6 (IL-6) using healthy controls from four nested case-control studies in the Nurses' Health Study cohort. Findings from this third study have the potential to provide evidence for the role of PM-related inflammation in cerebrovascular and other cardiovascular diseases. As part of study 3, we also examined whether there were differences in the associations by PM size fraction, exposure averaging time, or by smoking status.

Innovation

This series of studies addresses several research gaps identified in the current literature. A major strength of our HPFS study is the use of high quality outcome and covariate data. Many prior studies of short-term PM exposure and stroke risk are based on administrative data, where frequent misclassification of stroke events can bias effect estimates towards the null (Johnsen et al. 2002; Reker et al. 2002). Additionally, studies relying on administrative data rarely have access to information on variables outside of age or gender that may modify the effect. Our study is one of few to examine potential effect modification by clinically relevant variables. Another major strength of this study is the use of a nationwide kriging model to estimate exposure to ambient PM which may reduce exposure misclassification. Our second study of Maryland hospitalizations has complementary strengths. While this study was within a dataset of hospitalizations, the large number of cases in this study allowed for sufficient examination of risk within population subgroups, even for hemorrhagic stroke events, where previous analyses have often been under-powered. Both studies examining the association between PM exposures and stroke control of all time-invariant confounders through the case-crossover study design. While individual characteristics like gender and age are perfectly matched and controlled for, other factors such as obesity and hypertension are also controlled due to the relatively short windows between case and control periods. Additionally, we have selected a time-stratified design that controls for potential confounding by day of the

week and seasonality. Our third study examines the association between PM exposures and biomarkers of inflammation, which may shed light onto the pathophysiologic mechanisms that lead from PM exposures to cerebrovascular or other cardiovascular outcomes. Few previous studies have looked at associations between PM exposures and biomarkers of inflammation in large cohorts with well-characterized information on potential confounders. Reliable information on potential confounding variables is critical in such studies as certain inflammatory biomarkers of interest can be associated with cardiovascular risk factors outside of clinical disease. Our use of robust and validated spatiotemporal statistical models may capture more spatial variability in exposure estimates than the use of estimates from nearest or central monitoring locations. Additionally, the use of a nationwide cohort and exposure models extends the geographic scope of included subjects beyond studies limited to small regions or individual cities.

Chapter 2: Methods

This chapter serves as a supplement to the methods for Chapters 3-5

Study Design

Case-crossover Design

The study design for papers 1 and 2 was a time-stratified case-crossover design. The case-crossover design was proposed in 1991 by Maclure to study short-term transient effects on the risk of acute events (Maclure 1991). In this design, the cases are the only participants and the exposure-disease effect estimate is determined by comparing exposure just prior to the event during a 'hazard' or 'case' period to exposure in a 'control' or 'referent' period. One major advantage to this study design is the control-by-design of time invariant or time insensitive risk factors such as age, gender, and socioeconomic status, since each case act as their own control. The design was originally proposed with a unidirectional reference period – meaning that the control or reference period always occurred before the hazard period. However, a variety of exposures have subsequently been considered for use in case-crossover studies, and other strategies have been developed to control for bias that might arise due to the chosen reference strategy. Navidi noted that unidirectional control periods can lead to bias under certain situations and proposed using bidirectional designs to account for exposures with time trends (Navidi 1998). This design is possible when the outcome does not affect subsequent exposure and theoretically sound when a single event does not necessarily preclude the study subjects from an additional event

(Bateson and Schwartz 1999). Sampling symmetrically from times before and after the outcome also has the effect of expanding the temporal sampling frame while keeping the distance between the control and hazard period the same (Fung et al. 2003). Typically, multiple referents are chosen per case which also increases study power over a single unidirectional referent group. Simulation analyses have demonstrated that symmetric bidirectional designs are more resistant to confounding by time trends (Navidi 1998). Another reference strategy design that is used often is the time-stratified bidirectional design. With this strategy, referent periods are chosen from within predefined time-strata, but there are no set patterns in the placement of referents around the case period. A month-long stratum length is often used, as this allows multiple reference periods even after matching by day of the week. This reference strategy has been used heavily in the air pollution literature (Carracedo-Martínez et al. 2010).

We used the time-stratified case-crossover design to assess the association between the risk of stroke onset and PM levels for studies 1 and 2. Control periods were chosen using the time-stratified approach. Exposures during the case period were compared to exposures on the same day of the week and in the same calendar month as the case period (Levy et al. 2001; Lumley and Levy 2000). Since individual events do not affect the distribution of future exposure in the overall population, the use of control periods from both before and after the case occurrence is appropriate. (Maclure and Mittleman 2008). This design has been shown to be effective in controlling for seasonality, overall time-trends, as well as persistent and slow-varying confounders (Bateson and Schwartz 1999).

Nested Cohort

Our third study is a hybrid, non-classic design that uses controls from four nested case-control studies within the Nurses' Health Study cohort. Monthly PM exposure estimates with cumulative exposures up to a year prior to the month of blood draw were used in this retrospective analysis. As our exposure estimates occur before our measured outcome variables (biomarkers) and we are using only a limited sample of the full NHS cohort, the main study could best be considered a retrospective nested cohort. Our distance to road analysis is most appropriately categorized as a cross-sectional analysis.

Potential Biases

Selection Bias

We do not see selection bias as a major source of bias in our studies, but there are a few key issues worth discussion. Both study 1 and study 3 take place within well-defined cohorts of health professionals (HPFS, NHS, respectively). These occupational cohorts had eligibility criteria based on age, and occupational registration. One of the most limiting factors in the use of occupational cohorts is the external validity or generalizability when comparing to general national population. We discuss this further in the limitations section of this chapter. As we have used nonstandard designs within these cohorts and rely upon modeled exposure levels based on address (with high match rate), general problems of selection bias in cohort studies (differential non-response or study attrition by exposure status) are not as problematic as they might be otherwise. Our case-crossover study of MD

hospitalizations may also have issues with external generalizability from case selection as it does not capture fatal stroke cases. It such a scenario it is important that we refer to hospitalization risk for stroke instead of total stroke risk. It is also not perfectly reflective of stroke hospitalization for MD residents, as a small proportion of residents may have been transferred to a hospital out of state. Another potential issue related to selection bias for this study is the potential differentiation in stroke hospitalizations by urbanicity (rural vs. urban). Persons afflicted with stroke in rural areas may be less likely to be hospitalized after the stroke event due to increased risk of death as a result of higher travel times (Moy 2017). We also expect a differentiation in exposure levels and composition between rural and urban areas as traffic-density and frequency of industrial point sources vary between urban and rural areas (Kundu and Stone 2014). However, as this is a case-crossover design and exposure during the case period of an individual from a rural area would be compared to exposure during control periods of an individual from the same rural area, we would not necessarily expect changes in the effect estimates. Instead, selection bias in this manner further limits the geographic generalizability of the study.

Information Bias

Each of the three studies use modeled air pollution estimates as exposure variables. This deserves some broader discussion as it relates to exposure error and information bias. In each study, the models we have used estimate ambient pollution levels either at the individual's reported address or at the county-level where they reside. However, an individual's total exposure to PM includes their exposure to both outdoor and indoor particles. If it were possible to easily capture, obtaining each

individual's total personal exposure levels would yield more accurate effect estimates. However, assessing total personal exposure through the use of personal monitors is impractical for most large study designs or for examining longer exposures. Instead, most large epidemiological studies have relied on the use of ambient pollution estimates, which have been shown to correlate relatively well with personal exposures (Avery et al. 2010). Additionally, using ambient exposure levels to study health effects can be informative as it relates to possible interventions, as regulatory agencies, like the Environmental Protection Agency, set outdoor air quality standards.

In addition to the measurement error corresponding to the difference between total personal exposure and ambient exposure, it is also important to consider measurement error between the measured and true ambient pollution concentrations. In general, two types of error are usually discussed when discussing ambient pollution exposure estimate errors (Armstrong 1998; Sheppard et al. 2004; Zeger et al. 2000). The first is classical error, in which measurements vary about the true concentrations. Measurement errors in ambient pollution monitors could be considered classical error; as repeated individual measurements may vary slightly about the true concentration. The second type of error is Berkson error, in which the same approximate exposure is used for many subjects, and the true exposures vary about this approximation but have a mean equal to it (Armstrong 1998). The distinction is important. It has been shown that while classical error, if non-differential, has a typical effect of biasing estimates towards the null, a purely Berkson error yields

unbiased effect estimates (Armstrong 1998). In reality, however, most estimates of ambient pollution exposures have elements of both types of error, and they are often quite difficult to disentangle (Zeger et al. 2000). For example, in our second study of the association between stroke hospitalizations and $PM_{2.5}$, a modeled county-level PM estimate was used as a proxy for individual ambient exposure. Assuming this county-level estimate represented a perfect average of all residents in the county, we might expect this exposure estimate to have a mostly Berkson-type error leading to unbiased effect estimates. However, this assumption is unlikely to be true. Firstly, some error in the modeled estimate is likely. Secondly, even if the county-level were modeled to spatially represent a true average for the county, due to clustering in residential communities, it is unlikely to represent an average for participants of the county. Thus, the misclassification error in this study could be problematic and exceed the error from the studies in which we used modeled estimates at a participant's reported address.

There are also concerns about information bias using ICD-9 coding framework for stroke hospitalizations. Several studies have identified that coding of stroke cases and subtypes by ICD-9 codes is not always reliable, as they depend both on the expertise of the clerical staff and the accuracy of the medical records (Kokotailo and Hill 2005; Reker et al. 2002). As we expect these errors to be non-differential with respect to exposure, a bias towards the null would be expected. Another limitation is that we were not able to separate first stroke hospitalizations from recurrent hospitalizations in study 2. This is problematic if a patient had multiple stroke events in a short time period, as reference or control periods at times before and after the event would no

longer be appropriate. Another limitation of hospitalization data is the potential misclassification in exposure that occurs due to the time differential between stroke onset and hospitalization. This form of misclassification has also been shown to bias effect estimates towards the null (Lokken et al. 2009).

Confounding

Our two case-crossover studies control for time-invariant confounders by design. All personal characteristics that might be related to exposure and stroke are controlled by matching case and control periods by the individual within a single-month timeframe (the strata). However, variables that could vary over the course of the month and are associated with both PM and stroke should be considered potential confounders. Weather-related covariates are most likely to fit this description. Most studies have considered ambient temperature as a potential confounding variable in the association between transient levels PM and stroke. Treatment as a confounder is justified as prior studies have shown relationships between ambient temperature and PM and between temperature and stroke (Lian et al. 2015; Tai et al. 2010). Several studies have looked in detail at associations between ambient temperature and short-term stroke risks. A recent meta-analysis and review concluded that there was enough evidence to conclude that short-term changes in both low and high temperatures had significant results on cerebrovascular events (Lian et al. 2015). The overall pooled results from 20 studies showed for hot temperatures (defined as 75^{th}-99^{th} range of temperatures), a 1 degree increase resulted in a 1.13 percent increase in risk of stroke (95% CI: 0.58-1.68). In the range of cold temperatures (1^{st}-25^{th} distribution), a 1 degree Celsius decrease resulted in a 1.20% increase (95% CI: 0.84-1.57) in stroke

(Lian et al. 2015). We controlled for temperature with non-linear spline regression to account for these non-linear associations.

In our third study, potential confounding variables included variables matched by the individual nested case-control studies as well as other variables chosen *a priori*. Potential confounders included: meteorological season, age (months), BMI (kg/m^2) , smoking status (never, past, current), pack-years smoked, physical activity (missing, <3, 3 to <18, ≥18 metabolic equivalent hours/week), hypertensive status (yes or no self-response to physician diagnosis), use of postmenopausal hormones (nonuser, current user, premenopausal, unknown), census tract median household income, alcohol consumption (missing, 0g/day, ≥0.1 g/day), family history of myocardial infarction (yes or no), and diet (measured by Alternate Healthy Eating Index (Chiuve et al. 2012)). Although we have controlled for an extensive set of variables that may act as confounders in this study, minor errors in the self-reporting of these variables could contribute to residual confounding. Though we cannot definitely rule out error of this type, we expect any residual confounding to be minimal as self-reporting of health-related conditions in this cohort have been shown to be quite accurate (Colditz et al. 1986). For example, 99% and 85.7% of a sample of self-reports of hypertension, and high cholesterol in this cohort were validated in a previous study (Colditz et al. 1986).

Statistical Approach

Case-crossover analysis

This section briefly outlines the statistical approach for testing the proposed hypotheses in order to inform this supplemental methods chapter. A more detailed methodology is described in the chapter for each study. Though the variables differ, the statistical approach for both case-crossover studies was similar. Conditional logistic regression models were used to obtain estimates of odds ratios (ORs) and 95% confidence intervals (CIs) associated with an interquartile range (IQR) increase in $PM_{2.5}$ or PM_{10}. PM exposures on the day of the stroke event (lag 0), as well as exposures from 1 to 3 days previous to the stroke event (lag 1 to lag 3) were considered in separate models. Stratified models by each of the potential effect modifiers were also conducted.

Linear regression analysis

Multivariable linear regression models were used to test the hypothesis that PM exposures are associated with inflammatory biomarkers. Potential confounding variables were incorporated into the models in two stages. First crude models (basic) adjusted for variables which were matched in the individual nested case-control studies. Next, other established confounders and potential confounders were added to fully adjusted models. Results from linear regression analyses of the PM fraction models were presented per IQR in PM and as percent difference for each biomarker along with the corresponding 95% confidence interval. The presentation of linear regression effect estimates as percent difference was done to ease interpretation and

provide consistency across multiple biomarkers. Since the biomarker values have been log-transformed, percent difference was calculated by exponentiating both sides of the linear regression equation, so that the exponentiated regression coefficient represents the ratio change of the biomarker on the unlogged scale per IQR increase in PM. Basic and fully adjusted distance to road models included two exposure terms corresponding to nearest distance to a major roadway (0-49m, and 50-199m). Thus, results from distance to road models are presented as percent difference in the biomarker concentration in comparison to concentrations among those living greater than 200m from a major road. In order to test for effect modification by smoking, interaction terms for each smoking category (never, former, current, missing) were included in separate regression models.

Assessment of Effect Modification

Effect Modification in the Case-crossover studies

Personal characteristics are controlled for in a case-crossover study by design. With our month-long strata, any variable that does not change within a short time period (a single month) is matched by the selection of case and control exposure periods. However, effect modification by these variables can be examined. Though interaction terms can be used, most previous case-crossover studies have assessed effect modification of time-invariant variables with stratified models. As noted by Zanobetti et al., one reason to use stratification over interaction terms is the possibility that the effect modifier could modify associations with other covariates in addition to the exposure variable (Zanobetti and Schwartz 2005). A stratified analysis controls for

this. To test for the significance of effect modification with stratified models, we used chi-square tests of heterogeneity for testing significance between the model estimates as done by several previous studies (Montresor-López et al. 2015; Zanobetti and Schwartz 2005). To do this, we used the formula:

$$H = \sum_{i=1}^{I} w_i \left[\log(\hat{RR}_i) - \log \hat{RR} \right]^2 = \sum_{i=1}^{I} \frac{\left[\log(\hat{RR}_i) - \log \hat{RR} \right]^2}{\hat{Var}_i \left[\log(\hat{RR}_i) \right]}$$

Where: log (RR_i) is the natural log of the stratum-specific OR for stratum i, and log (RR) is the natural log of the overall adjusted OR when the data are pooled. Var (log(RR_i)) is the variance of the stratum-specifc log(OR) for stratum i. The test statistic is distributed as a Chi-square on i-1 degrees of freedom.

Effect Modification in the Nested Cohort

We tested for effect modification by smoking status in our third study by the use of interaction terms in our multivariable linear regression models. The smoking categories used were never smoker, former smoker, current smoker, and missing status. Thus, testing for effect modification by smoking status required the inclusion of 3 interaction terms. Significance testing was then determined by likelihood ratio tests between interaction and non-interaction models. For this study, we found no evidence of effect modification by smoking status. Because of this, the results from stratified models were not presented.

Model Specification

Study 1

Conditional logistic regression models were used to test the association between short-term PM exposures and stroke for study 1. All models controlled for mean daily temperature and were repeated for lag 0, 1, 2, 3 lag days as well as an average of exposure from lag day 0 to lag day 3. The model sets proceeded as follows: 1) overall stroke 2) stratified by ischemic or hemorrhagic stroke 3) stratified by non-fatal and fatal stroke outcome 4) limited to ischemic strokes and stratified by either age category, BMI category, smoking category, hypertension status, high cholesterol status, or current regular aspirin use 5) a sensitivity analysis limited to ischemic strokes and stratified by meteorological season (winter, spring, summer, fall).

Study 2

A series of stratified conditional logistic regression models were conducted for study 2 to test the association between short-term $PM_{2.5}$ exposure and stroke hospitalizations. All models controlled for maximum daily temperature and were repeated for lag 0, 1, 2, and 3 lag days. The model sets proceeded as follows: 1) overall cerebrovascular hospitalizations 2) stratified by ischemic, hemorrhagic, or 'other' stroke subtype 3) stratified by stroke subtype and cold (Oct-Apr) or warm (May-Sep) season 4) stratified by stroke subtype and either age category, gender, race/ethnicity 5) a sensitivity analysis stratified by stroke subtype and meteorological season (winter, spring, summer, fall).

Study 3

Multivariable linear regression models were used for study 3 to test the associations between PM exposures and inflammatory biomarkers. Separate models were conducted for each of the 4 biomarkers and each of the three PM exposure averaging times and three PM size fractions. Potential confounding variables were incorporated into the models in two stages. First crude models (basic) adjusted for variables which were matched in the individual nested case-control studies and included: meteorological season, age (months), BMI (kg/m^2) , smoking status (never, past, current), pack-years smoked, physical activity (missing, <3, 3 to <18, ≥18 metabolic equivalent hours/week), hypertensive status (yes or no self-response to physician diagnosis), use of postmenopausal hormones (nonuser, current user, premenopausal, unknown), census tract median household income, alcohol consumption (missing, 0g/day, ≥0.1 g/day), family history of myocardial infarction (MI) (yes or no), and diet (measured by Alternate Healthy Eating Index (Chiuve et al. 2012)). Continuous terms were used for PM (exposure), age, BMI, median household income, and pack-years smoked. Binary (2 categories) or dummy variables (more than 2 categories) were used for the other confounders and distance to road variables. Interaction terms were included in initial PM models and distance to road models, but removed in final models, as tests of effect modification were non-significant.

Model Assumptions

Logistic Regression and Splines

Our first two papers use conditional logistic regression models to test the hypotheses. One of the main assumptions of logistic regression models is the linearity of

independent variables and log odds. If the independent variables are not linearly related to the log odds, the test may underestimate the strength of the relationship and the relationship could be falsely rejected as non-significant. As a preliminary step to these models, we verified the assumption of linearity in the PM and temperature variables (the two continuous independent variables) by using likelihood ratio tests between models with linear continuous variables as well as models using nonlinear restricted cubic splines. No significant nonlinear relationships were found ($p>0.05$, data not shown).

Multivariable Linear Regression

Multivariable linear regression has several assumptions that need consideration during the modeling process. Variables must be normally distributed. The biomarker measurements used in this study, and generally in most similar studies, were right-skewed. To correct for non-normality, we log-transformed the biomarker distributions. Improvements in normality of the distributions of biomarkers were apparent (See Figure 1 for Example of CRP).

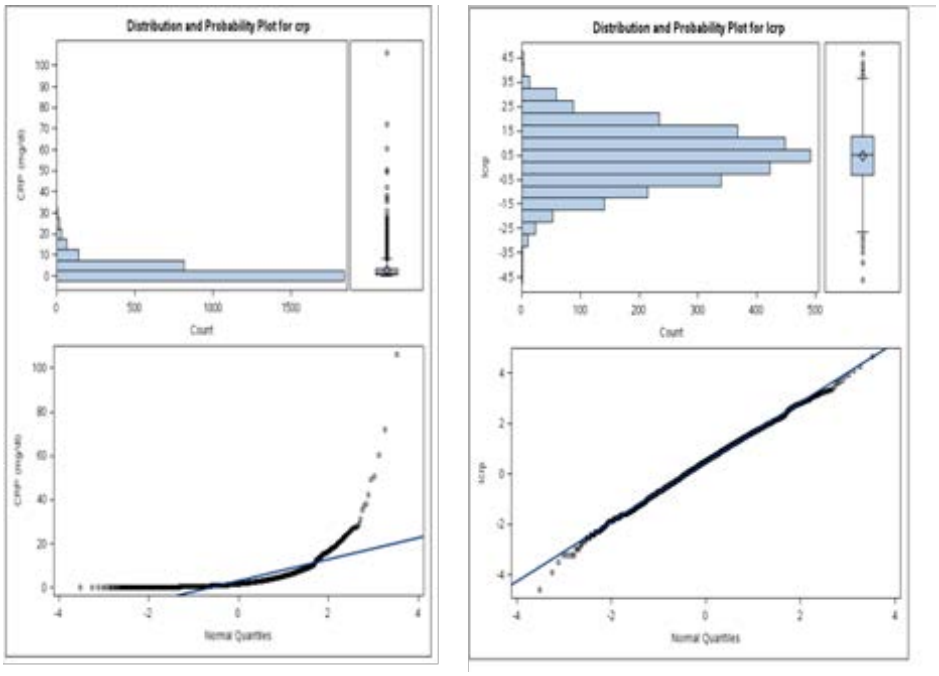

Figure 1. Histograms, box-plots, and normal quantile plots of unlogged CRP (left) and log-transformed CRP (right) distributions. The log-transformed distribution showed a higher degree of normality.

We next examined regression models for points of undue influence (outliers). We chose to address this through the use of quantile regression. This technique plots the regression coefficients by the chosen quantiles of the dependent variable (biomarker measurements). If the parameter estimate were not unduly influenced by certain biomarker values compared to others, we would expect the parameter estimates to be equivalent for each quantile. In this examination, we found deviations in the parameter estimates among high levels of biomarker values. Among the highest quantiles of biomarker distributions (~95%), parameter estimates for PM were depressed and had wide confidence intervals. This finding was used as justification to trim the upper tail of the biomarker distributions. As an example, a comparison of quantile regression plots before and after the outlier removal is presented as Figure 2.

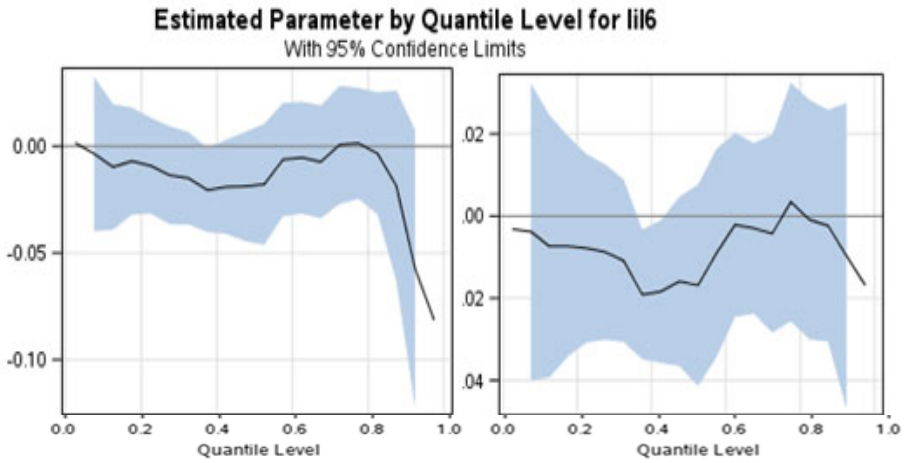

Figure 2. Quantile regression plot of parameter estimates for 12-month PM_{10} by logged IL-6 quantile both before (left) and after (right) upper-tail outlier removal (less movement in the parameter estimate indicates more appropriate model – note differences in scale)

Another assumption of multivariable linear regression is that independent variables are not highly correlated with each other. We tested for multicollinearity using the Variance Inflation Factor (VIF) statistic in SAS. Though we found high VIF values among the smoking category variables and the pack-yrs smoked variable, no other variable pairs showed VIFs of concern. None of the control variables showed high VIFs with the exposure variable.

Chapter 3: Case-crossover Analysis of Short-term Particulate Matter Exposures and Stroke in the Health Professionals Follow-up Study

Title Page

Case-crossover Analysis of Short-term Particulate Matter Exposures and Stroke in the Health Professionals Follow-up Study

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Abstract

Background: Stroke is a leading cause of morbidity and mortality in the United States. Associations between short-term particulate matter (PM) air pollution exposure and stroke are inconsistent. Many prior studies have used administrative and hospitalization databases where outcome misclassification is frequent and problematic.

Methods: In this case-crossover study, we used a nationwide kriging model to examine short-term ambient exposure to PM_{10} and $PM_{2.5}$ and risk of ischemic and hemorrhagic stroke among participants of the Health Professionals Follow-up Study. Conditional logistic regression models were used to obtain estimates of odds ratios and 95% confidence intervals associated with an interquartile range increase in $PM_{2.5}$ or PM_{10}. PM exposures from 0 to 3 lag days as well as a 4-day average of PM exposure were examined.

Results: We found positive significant associations between PM_{10} and ischemic stroke events in the HPFS cohort ($OR_{lag0\text{-}3}$=1.27; 95% CI: 1.03-1.56 per 14.58μg/m^3 increase), and associations were elevated for nonsmokers, aspirin nonusers, and those having never had a diagnosis of high cholesterol. In contrast, we found no evidence of a positive association between short-term exposure to PM and hemorrhagic stroke or between $PM_{2.5}$ and ischemic stroke in this cohort. Findings were relatively consistent across lag periods.

Conclusions: Our study provides evidence that ischemic and hemorrhagic strokes are heterogeneous outcomes and should be treated as such in analyses related to air pollution. Our study also adds to previous results that show PM exposure may increase the risk of ischemic stroke and adds the finding that those of previously

unidentified cardiovascular risk may be at elevated risk for acute PM-related ischemic stroke events.

Target Journals: 1) Environmental Health Perspectives 2) Environmental Health 3) Science of the Total Environment

Keywords: Particulate matter, stroke, air pollution, case-crossover

Introduction

Stroke is a leading cause of morbidity and mortality in the United States with 795,000 people experiencing a new or recurrent stroke every year (Benjamin et al. 2017). Of these, approximately 130,000 result in death, making stroke the fifth leading cause of death in the United States (Benjamin et al. 2017; CDC 2016b).

While a large and growing body of research has established the role of ambient exposure to air pollution and cardiovascular mortality and morbidity (Brook et al. 2010), the evidence linking particulate matter (PM) air pollution exposures to the risk of stroke remains equivocal (Ljungman and Mittleman 2014). In the epidemiologic literature, there have been two approaches to examine this relationship: studies of long-term PM exposure as a contributor to higher incidence of stroke and studies of short-term PM exposure as an acute trigger of stroke (Ljungman and Mittleman 2014; Maheswaran 2016). Prior studies of short-term PM exposure and stroke have provided inconsistent results, with some (Dominici et al. 2006; Wellenius GA et al. 2012; Zanobetti and Schwartz 2009) but not all (Anderson et al. 2001; O'Donnell et al. 2011) studies finding evidence of increased risk.

A major limitation of much of the prior research in this domain has been the reliance on administrative and hospitalization databases where outcome misclassification is frequent and problematic (Reker et al. 2002) and where ischemic and hemorrhagic strokes are often combined as a single outcome, despite differing pathophysiological pathways (Ljungman and Mittleman 2014). Additionally, exposure misclassification

in analyses relying on administrative databases can bias the results towards the null by up to 60%, as the timing of the event and the hospitalization are not always congruent (Lokken et al. 2009). For these reasons, a recent review has urged for additional studies using cohort or registry data with well-classified and medically-reviewed outcome data (Wang et al. 2014).

In this case-crossover study, we used a nationwide kriging model to examine short-term ambient exposure to PM_{10} and $PM_{2.5}$ and risk of ischemic and hemorrhagic stroke among participants of the Health Professionals Follow-up Study (HPFS) cohort. Reported strokes were medically-reviewed to ensure accuracy and to obtain the exact date of the stroke event. Effect modification by several clinically-relevant variables was also examined.

Methods

Study population

The HPFS is an ongoing, prospective cohort study of 51,529 men who were 40–75 years of age at baseline in 1986 (HPFS 2011). Participants were dentists, pharmacists, optometrists, osteopath physicians, podiatrists, or veterinarians at the time of enrollment. Study participants receive mailed questionnaires with questions about disease, medical history, and health-related risks and behaviors every two years. Mailing addresses were primarily residential, but some occupational addresses were included as mailing addresses for the cohort. Response rates to questionnaires are generally greater than 90%. Our case-crossover study population included all

participants who experienced a stroke between 1999 and 2010, the dates which PM and temperature data were available. Cases were included if both an exact date of the stroke event was known and the participant's addresses on the questionnaire immediately before the stroke event was in the conterminous US. The HPFS is sponsored by the Harvard School of Public Health and is funded by the National Cancer Institute and was approved by the Harvard School of Public Health IRB.

Outcome Assessment

Self-reported strokes were adjudicated by trained study physicians reviewing medical records using a standardized approach. Strokes were confirmed when medical records documented a neurologic deficit with sudden or rapid onset persisting for >24 hours without evidence for other causes, unless death supervenes or there is a demonstrable lesion compatible with acute stroke on brain imaging studies. Strokes were classified as ischemic, hemorrhagic, or of undetermined type and the exact date of the stroke event was noted. Death events were ascertained via communication with proxy respondents and/or National Death Index searches.

Exposure Assessment

Previously, validated national-scale, log-normal ordinary kriging models for $PM_{2.5}$ and PM_{10} have been attached to the addresses of all HPFS participants. These models are explained in detail elsewhere (Liao et al. 2006). Briefly, all ambient PM data recorded at monitors operating in the contiguous United States during the study period, 1999-2010 were obtained from the U.S. Environmental Protection Agency Air Quality System (AQS) for PM_{10} and $PM_{2.5}$. The authors used a semiautomated program built on ArcView GIS (version 8.3) software and its Geostatistical Analyst

extension (ESRI, Inc. Redlands, CA) to produce kriging-estimated daily mean concentrations at each geocoded participant address. The program relied on a spherical model to perform log-normal ordinary kriging at national-scale and the weighted least-squares method to estimate semivariograms. From these models, $PM_{2.5}$ and PM_{10} estimates were available for each day from January 1, 1999 to December 31, 2010.

Covariates

Temperature was considered as a potential confounder in this analysis, and daily mean temperature values were attached to each HPFS participant's geocoded address from 1999 to 2010. Data on air temperature were obtained from the Modern Era Retrospective-analysis for Research and Applications (MERRA) project (NASA 2017). MERRA data are available hourly on a grid across the continental US with an approximately 55 km cell size. Hourly gridded data were assigned local time and date based on the time zone of each grid point's location and then averaged by day. The daily averages were included in spatially-smoothed generalized additive models (GAMs) which were then used for space-time prediction of daily temperature values at any location in the conterminous United States.

Several variables which may modify the relationship between PM exposures and stroke were assessed as potential effect modifiers. These variables included: age (<70, ≥70 years old), body mass index (BMI) (< 25.0, < 30.0, or ≥ 30.0 kg/m2), smoking status (current, former, never), hypertensive status ('yes' or 'no' as to ever having been diagnosed by health professional), hypercholesterolemia ('yes' or 'no' as to ever

having been diagnosed by health professional), and current aspirin use ('yes' as 2+ tablets per week; 'no' otherwise). Responses were obtained from the most recent HPFS questionnaire prior to the stroke event.

Statistical Analysis

A time-stratified case-crossover study design was used in this study to analyze the association between exposure to ambient particulate matter and stroke events. Proposed by Maclure, the case-crossover design has increasingly been used to examine transient effects on the risk of acute events (Maclure 1991). With this design, each case's exposure just prior to the event is compared to exposure at other referent periods. In this way, each case serves as his/her own control, and confounding by invariant and slowly changing risk factors is controlled.

In order to prevent time-trend bias and to ensure unbiased conditional logistic regression estimates, we used a time-stratified approach for referent period selection (Janes et al. 2005). Month-long strata were used, and exposures during the case period (day of or days previous to stroke event) were compared to exposures on the same day of the week and in the same calendar month as the case period.

Using SAS 9.4 (SAS Institute Inc., Cary, NC), conditional logistic regression models were used to obtain estimates of odds ratios (ORs) and 95% confidence intervals (CIs) associated with an interquartile range increase in $PM_{2.5}$ or PM_{10}. PM exposures on the day of the stroke event (lag 0), as well as exposures from 1 to 3 days previous to the stroke event (lag 1 to lag 3) were considered in separate models. Additionally, a

4-day average of PM exposures (lag 0 to lag 3) was included to represent the total exposure to PM in the 4-day period. Days with missing estimates for PM concentrations were excluded from analysis.

Linear terms for mean daily temperature (°C) were included in each model as a control variable. Effect modification of the relationship between PM and ischemic stroke was examined by age, BMI, smoking status, hypercholesterolemia, hypertensive status, and regular aspirin use through stratified conditional logistic models. Significance testing between the stratified estimates was assessed using chi-square tests of model heterogeneity. Sensitivity analyses included the examination of effect by season, adjustment by temperature using restricted cubic splines, and the extension of lag days to 6 days before the stroke event.

Results

A total of 724 stroke cases were medically-reviewed and had a complete record of the date of event; 716 of these also had addresses in the conterminous US and were geocoded. Of the 716 cases used in this study, 537 (75.0%) were ischemic strokes, 127 (17.7%) were hemorrhagic stokes, and 58 (8.1%) were of undetermined type. Additionally, of the 716 stroke events, 162 were determined to be fatal and 553 were non-fatal.

PM exposures on the day of the event as well as descriptive characteristics are presented by total, ischemic, and hemorrhagic stroke cases in Table 1. Exposures to PM and outdoor ambient temperature were lower on the day of the event for hemorrhagic stroke than for ischemic stroke using two-sample independent t-tests

($p<0.01$). A higher proportion of cases with ischemic stroke were slightly younger, had higher BMIs, and were more likely to be current or former smokers, though none of these differences between ischemic and hemorrhagic stroke cases were statistically significant. Stroke events were more often fatal among hemorrhagic strokes (48.8%) than among ischemic strokes (10.4%). Over half of the total stroke cases reported having ever had hypertension (63.4%), having ever had high cholesterol (59.6%), or were regularly taking aspirin at the last survey before the stroke (59.2%). Proportions were similar between stroke subtypes for having ever reported having hypertension or being a current regular aspirin user. However, a higher proportion of ischemic stroke cases reported having ever had high cholesterol (61.6%) compared to reports from hemorrhagic stroke cases (49.6%).

ORs and 95% CIs between short-term PM exposures and total stroke, stroke subtype, and stroke outcome are presented in Table 2. On the day of the stroke event, the association between $PM_{2.5}$ and total stroke was OR=1.01 (95% CI: 0.90, 1.13), and between PM_{10} and total stroke, the association was OR=1.08 (95% CI: 0.95, 1.23). When all stroke events were considered, no significant associations were found between the PM exposures and total stroke for each of the 4 lag days or the 4-day cumulative lag period.

We found a significant positive association between PM_{10} and ischemic stroke using the 4-day cumulative lag exposure ($OR_{Lag0_3=}$1.27; 95% CI: 1.03-1.56), as well as suggestive positive associations on the day of (OR_{Lag0}=1.14; 95% CI: 0.99-1.31) and

day prior (OR_{Lag1}=1.14; 95% CI: 0.99-1.31) to the stroke event. Significant associations were also found between PM_{10} and the subset of nonfatal strokes on the day of stroke event (OR_{Lag0}=1.16; 95% CI: 1.01-1.34) and the 4-day cumulative lag period (OR_{Lag0_3}=1.27; 95% CI: 1.03-1.56). We found significantly negative results for $PM_{2.5}$ (OR=0.71; 95% CI: 0.51 – 0.99) and PM_{10} (OR=0.55; 95% CI: 0.37 – 0.82) in the subset of hemorrhagic stroke cases for lag day 1. Likewise, associations for fatal stroke in the same lag period were also negative, as over 50% of fatal outcomes were from hemorrhagic stroke events. No significant positive associations with $PM_{2.5}$ exposures were found by stroke subtype or stroke outcome. ORs for both $PM_{2.5}$ and PM_{10} were higher for ischemic strokes compared to hemorrhagic strokes and higher among nonfatal strokes compared to fatal strokes. However, in tests of heterogeneity, only associations for PM_{10} exposures by stroke subtype reached statistical significance in lag 1 models (OR_{isc}=1.14; 95% CI: 0.99-1.31 vs OR_{hem}=0.55; 95% CI: 0.37 -0.82; p<0.01) and lag0_3 models (OR_{isc}=1.27; 95% CI: 1.03-1.56 vs OR_{hem}=0.68; 95% CI: 0.42 -1.10; p=0.02).

ORs and 95% CIs between short-term PM exposures and ischemic stroke by age, BMI, and smoking status are presented in Table 3. No significant differences were found by age strata or by BMI categorizations for either PM_{10} or $PM_{2.5}$ exposures. Associations between PM and ischemic stroke were higher among nonsmokers than among former or never smokers. On lag day 0, the ORs for nonsmokers for both PM_{10} (OR=1.23; 95% CI: 0.98-1.55) and $PM_{2.5}$ (OR=1.20; 95% CI: 0.96-1.51) were elevated when compared to the ORs for former smokers (OR_{PM10}=1.08; 95% CI:

0.88-1.32; $OR_{PM2.5}$=0.98; 95% CI: 0.81-1.18) or never smokers (OR_{PM10}=0.92; 95% CI: 0.52-1.65; $OR_{PM2.5}$=0.69; 95% CI: 0.37-1.28). Though tests of heterogeneity did not reach statistical significance, this finding was consistent for both PM fractions and across all lag days, with the exception of PM_{10} lag day 1.

ORs and 95% CIs between short-term PM exposures and stroke by self-reported current or past hypertensive or hypercholesterolemia status and regular aspirin use are presented in Table 4. No significant differences were found by hypertension strata for either PM_{10} or $PM_{2.5}$ exposures. Elevated ORs were found among those that reported having never received a diagnosis of high cholesterol compared to those that had received a diagnosis. Differences were significant in the lag0-3 models for both PM_{10} (OR_{YES}=1.10; 95% CI: 0.84-1.43 vs. OR_{NO}=1.60; 95% CI: 1.15-2.23; p=0.03) and $PM_{2.5}$ (OR_{YES}=0.89; 95% CI: 0.72-1.11 vs. OR_{NO}=1.22; 95% CI: 0.91-1.63; p =0.04) models. Among those having reported no current regular aspirin usage, there was a significantly positive association between PM_{10} exposure and risk of stroke 3 days previous to the stroke event (OR=1.37; 95% CI: 1.06-1.76) and using the 4-day cumulative exposure (OR=1.56; 95% CI: 1.12-2.17). No significant associations between $PM_{2.5}$ and stroke were found among aspirin users or nonusers. However, the ORs for both PM_{10} and $PM_{2.5}$ were higher for nonusers compared to users across all lag periods, and these results reached statistical significance ($p<0.05$) in tests of heterogeneity for both PM fractions in the lag 3 and lag0-3 models. In sensitivity analyses, associations between PM_{10} or $PM_{2.5}$ and total stroke did not significantly

differ by season (Supplemental Table 1) or by the use of restricted cubic splines to control for temperature (Supplemental Table 2).

Discussion

We found positive significant associations between PM_{10} and ischemic and nonfatal stroke events. In contrast, we found no evidence of a positive association between short-term exposure to PM and hemorrhagic stroke or between $PM_{2.5}$ and ischemic stroke in this cohort. Associations between PM and ischemic stroke were elevated for nonsmokers, aspirin nonusers, and those having never had a diagnosis of high cholesterol. Findings were relatively consistent across lag periods.

While many studies have examined total cerebrovascular hospital admissions or mortality with PM, relatively few studies have examined the relationship between short-term PM exposure and ischemic and hemorrhagic strokes, specifically (Ljungman and Mittleman 2014; Wang et al. 2014). Our finding of a positive association between short-term PM_{10} exposure and ischemic stroke is consistent with several studies (Tsai et al. 2003; Wellenius et al. 2005; Wordley et al. 1997). Wellenius et al. examined a database of Medicare recipients in 9 U.S. cities. They found a small, but significant, increase in ischemic stroke admissions with increased city-wide PM_{10} concentrations on the day of admission (1.03% increase per interquartile range increase [23.0μg/m^3]; 95% CI: 0.04-2.04) (Wellenius et al. 2005). As in our study, they did not find positive associations between PM_{10} exposure and hemorrhagic stroke (Wellenius et al. 2005). Another study by Tsai et al of stroke admissions in Taiwan, found that on days of ≥20°C, an interquartile range increase

(66.3µg/m^3) in PM_{10} was associated with both ischemic (OR=1.46; 95% CI: 1.32-1.61) and hemorrhagic (OR=1.54; 95% CI: 1.31-1.81) stroke admissions (Tsai et al. 2003). The evidence between short-term exposure to PM_{10} and ischemic stroke is not consistent, however. Other studies have found no evidence of a relationship (Andersen et al. 2010; Chan et al. 2006; Henrotin et al. 2007; Mechtouff et al. 2012; Villeneuve et al. 2006).

Similarly, the existing literature for an association between $PM_{2.5}$ and stroke has been mixed, with some studies (Delfino et al. 2009a; Dominici et al. 2006; Lisabeth et al. 2008; Wellenius GA et al. 2012) but not others (Lippmann et al. 2000; Mechtouff et al. 2012; O'Donnell et al. 2011; Villeneuve et al. 2006) finding positive associations. Of these studies, only a few used medically reviewed stroke cases. Wellenius et al. (2012) performed medical review of 1,705 Boston area patient records to confirm ischemic stroke events. They estimated the OR of ischemic stroke to be 1.11 (95% CI: 1.03-1.20) (*P*=0.006) per interquartile range increase in $PM_{2.5}$ levels (6.4 µg/m^3) (Wellenius GA et al. 2012). In a multicenter cohort study in Lyon, France, Mechtouff et al. used a case-crossover design on 376 medically-reviewed stroke patients. The found no association between ischemic stroke and $PM_{2.5}$ (OR=0.97; 95% CI: 0.83-1.12 per SD [10.4µg/m^3] increase) (Mechtouff et al. 2012). Likewise, O'Donnell et al used data from a stroke registry in Canada and found no association with $PM_{2.5}$ among 9,202 ischemic stroke cases (O'Donnell et al. 2011).

Our findings of significantly negative results between PM and hemorrhagic stroke on the day prior to the stroke event were unexpected. Though a few prior studies have also reported significant negative associations between PM and stroke (Jalaludin et al. 2006; Talbott et al. 2014), such findings have been limited to certain lag periods, locations, seasons, or other subcategory of total stroke events. Our findings for hemorrhagic stroke were not consistent across lag periods and the sample of cases (n=121) was too small to effectively examine the relationship by other variables. These results require more investigation.

As most existing studies examining the short-term associations between stroke and particulate matter air pollution have been studies of administrative datasets, few have been able to examine individual clinical variables as potential effect modifiers of the relationship. Such findings are important as they may identify individuals who are more vulnerable to the effects of air pollution mediated stroke. Of the covariates we examined in this study, only age, which is available in many administrative datasets, has been tested widely as a potential effect modifier. Similar to our study, most of these studies found no substantial difference in the PM/stroke association by age group (Anderson et al. 2001; Barnett et al. 2006; Delfino et al. 2009a; Larrieu et al. 2007; Linn et al. 2000). Though our results did not reach statistical significance in tests of heterogeneity, we found consistently elevated ORs for both PM_{10} and $PM_{2.5}$ among nonsmokers. Only a few studies have examined smoking status as a potential effect modifier of the relationship between short-term PM exposure and stroke; each

finding no differences by smoking status strata (O'Donnell et al. 2011; Oudin et al. 2010).

To our knowledge, our study is also the first to demonstrate effect modification by current aspirin usage on the PM ischemic stroke relationship. Aspirin is an antiplatelet medication often given to those at elevated cardiovascular risk and is known to reduce the risk of ischemic stroke (Lei et al. 2016). In this study, we found elevated ORs for aspirin nonusers compared to users across all lag periods and both PM fractions, with several of those differences reaching statistical significance in heterogeneity tests. While we are unaware of other studies of aspirin as an effect modifier for PM and stroke, Villenauvue et al examined both current antiplatelet and anticoagulant medication use (medications unnamed) in an analysis of short-term NO_2 exposure and stroke (Villeneuve et al. 2012). They found no differences by antiplatelet use or nonuse, but found a significant association between NO_2 exposure and ischemic stroke when limited to anti-coagulant nonusers (Villeneuve et al. 2012). As aspirin is more likely to be taken regularly among those with higher levels of cardiovascular risk, our findings of potential effect modification by past diagnosis of high cholesterol is consistent with these results. Among ischemic stroke cases in our study, 69% and 72% of aspirin users reported having been diagnosed with high cholesterol and hypertension, respectively. Whether these results are evidence of a higher risk from PM-related stroke among those with little previously known cardiovascular risk, or whether potential medication use may lower or eliminate PM-related ischemic stroke risk is difficult to disentangle.

Our findings of elevated associations between PM and ischemic stroke among several subgroups not only helps to identify those potentially most vulnerable to PM-related stroke, but the findings may also shed light on pathophysiologic mechanisms by which exposure to PM may lead to stroke. Though the exact mechanisms are not yet fully understood, commonly hypothesized pathways include increased systemic inflammation and oxidative stress leading to procoagulant effects, autonomic irregularities, vascular endothelial dysfunction, and thrombosis which could enhance the likelihood of an ischemic event (Franchini and Mannucci 2007; Mills et al. 2007). Our finding of reduced associations among smokers might be understood in this context, as cigarette smoke influences and may saturate these same pathways (Barnoya and Glantz 2005). The specific role of platelet activation as a key mechanism of action has achieved recent attention with several studies showing an association between increased PM exposure and markers of platelet activation (Rich DQ et al. 2012; Wu et al. 2012). Of major interest to our study is a recent finding by Bacarerra et al (2016) that some of the effects of increased ambient PM on platelet function were mitigated when subjects were taking aspirin (Becerra et al. 2016).

A major strength of this study is the use of high quality outcome and covariate data from HPFS. Many prior studies of short-term PM exposure and stroke risk are based on administrative data, where frequent misclassification of stroke events can bias effect estimates towards the null (Johnsen et al. 2002; Reker et al. 2002). Additionally, studies relying on administrative data rarely have access to information

on variables outside of age or gender that may modify the effect. Our study is one of few to examine potential effect modification by clinically relevant variables. Another major strength of this study is the use of a nationwide kriging model to estimate exposure to ambient PM which may reduce exposure misclassification.

This study also has several limitations. First, although studies have shown ambient concentrations of PM correlate relatively well with personal exposures (Avery et al. 2010), it should also be noted that an individual's total exposure to PM represents the sum of their exposure to particles both of outdoor origin as well as particles of indoor origin. Thus, some exposure misclassification is unavoidable. Second, the timing of the stroke event compared to the timing of hospitalization or death is not always congruent. Though we examined potential effects over multiple lag periods, this source of misclassification has been shown to bias effect estimates towards the null (Lokken et al. 2009). Third, although these data came from a large cohort, low sample sizes in this case-only study for hemorrhagic stroke, and for several categories of effect modifiers were limiting. Finally, results of this study in the HPFS cohort may not be generalizable to younger men, women, those of non-Caucasian descent, or to a wide range of socioeconomic levels.

Conclusion

Our study provides more evidence that ischemic and hemorrhagic strokes are heterogeneous outcomes and should be treated as such in analyses related to air pollution. Our study also adds to previous results that show PM exposure may

increase the risk of ischemic stroke and adds the finding that those of previously unidentified cardiovascular risk may be at elevated risk for acute PM-related ischemic stroke events. Whether this is by means of managed risk by medication use or another mechanism requires more investigation. Findings from this study are in agreement with the last comprehensive review and assessment by the Environmental Procetion Agency that exposure to particulate matter is associated with stroke and other cardiovascular outcomes (US EPA 2009). As US EPA re-evaluates regulations on the criteria pollutants every five years under The Clean Air Act, results from these studies may directly contribute to future policy actions.

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Tables

Table 1. Characteristics and event day exposures of participants with total, ischemic, and hemorrhagic stroke from 1999 to 2010 in the Health Professionals Follow-up Study

	Total Stroke (n=716)	Ischemic Stroke (n=537)	Hemorrhagic Stroke (n=121)
	mean ± SD or N (%)	mean ± SD or N (%)	mean ± SD or N (%)
Stroke Outcome			
Fatal	126 (17.6%)	56 (10.4%)	59 (48.8%)*
Non-fatal	590 (82.4%)	481 (89.6%)	62 (51.2%)
Age (years)	76.1 ± 8.4	75.1 ± 8.3	77.3 ± 8.0*
BMI (kg/m^2)			
≤ 25	275 (38.4 %)	189 (35.2 %)	54 (44.6 %)
>25 to ≤ 30	340 (47.5 %)	268 (49.9 %)	52 (43.0 %)
>30	98 (13.7 %)	77 (14.3%)	15 (12.4 %)
Smoking status			
Never	255(35.6%)	188 (35.0%)	51 (42.1%)*
Former	343(47.9%)	272 (50.7%)	45 (37.2%)
Current	40 (5.6%)	32 (6.0%)	4 (3.3%)
Missing	78 (10.9%)	45 (8.4%)	21 (17.4%)
Ever Hypertension "Yes" [a]	454 (63.4%)	345 (64.3%)	75 (62.0%)
Ever High Cholesterol "Yes" [a]	427 (59.6%)	331 (61.6%)	60 (49.6%)*
Current Regular Aspirin Use "Yes" [a]	424 (59.2%)	321 (59.8%)	71 (58.7%)
$PM_{2.5}$ ($\mu g/m^3$)[b]	12.9 ± 7.5	13.1 ± 7.6	11.9 ± 6.7
PM_{10} ($\mu g/m^3$)[b]	26.3 ± 12.3	26.8 ± 12.7	23.6 ± 10.2*
Temperature (C)[b]	12.4 ± 10.4	12.9 ± 10.3	10.1 ± 11.2*

[a] Self-report from biennial questionnaire : Have you had a physician tell you that you have high blood pressure?"; "Have you had a physician tell you that you have high cholesterol?"; "Do you regularly take aspirin?", ("Yes" defined as 2+ tablets /week)

[b] Daily estimate on the day of the stroke event

* Denotes significance ($p<0.05$) between ischemic and hemorrhagic strokes in two-sample t-tests or Chi-square tests

Table 2. Odds Ratios and 95% Confidence Intervals of the Association of Short-term Particulate Matter Exposure with Total Stroke, Stroke Subtype, and Stroke Outcome in the Health Professionals Follow-up Study

	N	Lag 0 OR (95% CI)[a]	Lag 1 OR (95% CI)[a]	Lag 2 OR (95% CI)[a]	Lag 3 OR (95% CI)[a]	Lag 0-3 (Avg) OR (95% CI)[a]
Total Stroke	716					
$PM_{2.5}$		1.01 (0.90, 1.13)	0.93 (0.83, 1.05)	0.93 (0.83, 1.05)	1.01 (0.90, 1.13)	0.95 (0.81, 1.11)
PM_{10}		1.08 (0.95, 1.23)	1.05 (0.93, 1.19)	1.06 (0.93, 1.20)	1.10 (0.96, 1.25)	1.15 (0.96, 1.38)
Ischemic Stroke	537					
$PM_{2.5}$		1.02 (0.89, 1.16)	0.98 (0.87, 1.12)	0.95 (0.83, 1.09)	1.02 (0.90, 1.16)	1.00 (0.84, 1.19)
PM_{10}		1.14 (0.99, 1.31)	1.14 (0.99, 1.31)	1.11 (0.96, 1.28)	1.11 (0.95, 1.29)	1.27 (1.03, 1.56)
Hemorrhagic Stroke	121					
$PM_{2.5}$		0.88 (0.65, 1.20)	0.71 (0.51, 0.99)	1.01 (0.77, 1.32)	1.06 (0.81, 1.39)	0.84 (0.55, 1.26)
PM_{10}		0.84 (0.60, 1.18)	0.55 (0.37, 0.82)	0.85 (0.61, 1.19)	1.09 (0.80, 1.48)	0.68 (0.42, 1.10)
Nonfatal Stroke	563					
$PM_{2.5}$		1.06 (0.93, 1.20)	0.98 (0.86, 1.11)	0.98 (0.86, 1.12)	1.04 (0.92, 1.19)	1.04 (0.87, 1.24)
PM_{10}		1.16 (1.01, 1.34)	1.12 (0.98, 1.29)	1.11 (0.96, 1.28)	1.10 (0.95, 1.28)	1.27 (1.04, 1.56)
Fatal Stroke	153					
$PM_{2.5}$		0.86 (0.66, 1.11)	0.74 (0.56, 0.99)	0.77 (0.59, 1.00)	0.89 (0.69, 1.15)	0.70 (0.49, 1.01)
PM_{10}		0.80 (0.60, 1.07)	0.80 (0.59, 1.07)	0.89 (0.67, 1.19)	1.07 (0.81, 1.43)	0.79 (0.53, 1.19)

[a]ORs calculated from conditional logistic regression models and presented by change in PM IQR ($PM_{2.5IQR}$ =8.73; PM_{10IQR} = 14.58). All ORs control for mean daily temperature.

Table 3. Odds Ratios and 95% Confidence Intervals of the Association between Short-term Particulate Matter Exposure and Ischemic Stroke by Age, BMI, and Smoking Status Categories in the Health Professionals Follow-up Study

	N	Lag 0 OR (95% CI)[a]	Lag 1 OR (95% CI)[a]	Lag 2 OR (95% CI)[a]	Lag 3 OR (95% CI)[a]	Lag0-3 (Avg) OR (95% CI)[a]
$PM_{2.5}$						
Age <70	250	0.98 (0.81, 1.20)	0.96 (0.79, 1.16)	1.00 (0.81, 1.22)	1.07 (0.89, 1.29)	1.02 (0.78, 1.33)
Age ≥70	287	1.05 (0.87, 1.25)	1.00 (0.85, 1.19)	0.92 (0.77, 1.10)	0.98 (0.82, 1.18)	0.98 (0.78, 1.24)
p-value[b]		0.65	0.51	0.46	0.20	0.55
PM_{10}						
Age <70	250	1.18 (0.96, 1.47)	1.15 (0.93, 1.41)	1.20 (0.97, 1.48)	1.13 (0.92, 1.40)	1.38 (1.01, 1.88)
Age ≥70	287	1.10 (0.91, 1.33)	1.13 (0.94, 1.38)	1.04 (0.85, 1.27)	1.08 (0.87, 1.34)	1.18 (0.90, 1.56)
p-value[b]		0.62	0.92	0.30	0.75	0.14
$PM_{2.5}$						
BMI < 25	189	1.01 (0.82, 1.26)	0.95 (0.78, 1.15)	0.89 (0.72, 1.10)	0.96 (0.77, 1.19)	0.93 (0.70, 1.22)
BMI ≥ 25 to <30	268	1.04 (0.85, 1.26)	1.05 (0.86, 1.29)	1.01 (0.82, 1.24)	1.02 (0.84, 1.23)	1.05 (0.80, 1.38)
BMI ≥ 30	77	0.92 (0.65, 1.31)	0.91 (0.67, 1.24)	0.95 (0.69, 1.32)	1.17 (0.84, 1.64)	0.98 (0.64, 1.50)
p-value[b]		0.85	0.67	0.70	0.60	0.81
PM_{10}						
BMI < 25	189	1.07 (0.85, 1.36)	1.02 (0.79, 1.31)	1.07 (0.83, 1.38)	1.05 (0.81, 1.36)	1.14 (0.79, 1.64)
BMI ≥ 25 to < 30	268	1.21 (0.99, 1.47)	1.26 (1.03, 1.54)	1.18 (0.97, 1.43)	1.08 (0.87, 1.35)	1.38 (1.03, 1.84)
BMI ≥ 30	77	0.96 (0.65, 1.43)	1.04 (0.75, 1.44)	0.96 (0.66, 1.40)	1.35 (0.94, 1.95)	1.15 (0.69, 1.92)
p-value[b]		0.53	0.35	0.62	0.51	0.68
$PM_{2.5}$						
Never Smokers	188	1.20 (0.96, 1.51)	1.11 (0.92, 1.34)	1.08 (0.87, 1.35)	1.14 (0.94, 1.40)	1.23 (0.94, 1.62)
Former Smokers	272	0.98 (0.81, 1.18)	0.86 (0.71, 1.04)	0.90 (0.74, 1.10)	0.92 (0.76, 1.12)	0.86 (0.66, 1.12)
Current Smokers	32	0.69 (0.37, 1.28)	0.87 (0.48, 1.61)	0.80 (0.45, 1.43)	0.90 (0.53, 1.53)	0.72 (0.34, 1.52)
p-value[b]		0.15	0.15	0.39	0.29	0.12
PM_{10}						
Never Smokers	188	1.23 (0.98, 1.55)	1.18 (0.92, 1.50)	1.28 (0.98, 1.69)	1.62 (1.13, 2.33)	1.23 (0.98, 1.55)
Former Smokers	272	1.08 (0.88, 1.32)	0.95 (0.77, 1.17)	1.06 (0.86, 1.31)	1.12 (0.83, 1.50)	1.08 (0.88, 1.32)
Current Smokers	32	0.92 (0.52, 1.65)	1.82 (1.04, 3.17)	0.91 (0.49, 1.68)	1.17 (0.52, 2.64)	0.92 (0.52, 1.65)
p-value[b]		0.14	0.55	0.07	0.43	0.28

[a]ORs calculated from conditional logistic regression models and presented by change in PM IQR ($PM_{2.5IQR}$ =8.73; PM_{10IQR} = 14.58). All ORs control for mean daily temperature. [b]p-value from Chi-sq tests of model heterogeneity

Table 4. Odds Ratios and 95% Confidence Intervals of the Association between Short-term Particulate Matter Exposure and Ischemic Stroke by Current or Past Hypertensive and Hypercholesterolemia Status and Current Regular Aspirin Use Categories in the Health Professionals Follow-up Study

	N	Lag 0 OR (95% CI)[a]	Lag 1 OR (95% CI)[a]	Lag 2 OR (95% CI)[a]	Lag 3 OR (95% CI)[a]	Lag0-3 (Avg) OR (95% CI)[a]
Ever Hypertension[b]						
$PM_{2.5}$						
Yes	345	0.96 (0.82, 1.13)	0.95 (0.82, 1.11)	0.92 (0.79, 1.09)	0.98 (0.83, 1.14)	0.93 (0.76, 1.15)
No	192	1.14 (0.90, 1.44)	1.07 (0.84, 1.36)	1.02 (0.80, 1.29)	1.14 (0.90, 1.45)	1.17 (0.84, 1.63)
p-value[c]		0.25	0.57	0.42	0.13	0.13
PM_{10}						
Yes	345	1.08 (0.91, 1.29)	1.05 (0.88, 1.25)	1.10 (0.92, 1.32)	1.09 (0.90, 1.32)	1.18 (0.92, 1.52)
No	192	1.25 (0.98, 1.60)	1.34 (1.05, 1.70)	1.11 (0.87, 1.41)	1.13 (0.88, 1.45)	1.46 (1.02, 2.09)
p-value[c]		0.35	0.10	0.69	0.85	0.11
Ever High Cholesterol[b]						
$PM_{2.5}$						
Yes	331	0.94 (0.79, 1.10)	0.90 (0.77, 1.06)	0.89 (0.75, 1.05)	1.03 (0.87, 1.22)	0.89 (0.72, 1.11)
No	206	1.21 (0.96, 1.53)	1.18 (0.95, 1.46)	1.09 (0.87, 1.36)	1.00 (0.81, 1.23)	1.22 (0.91, 1.63)
p-value[c]		0.07	0.09	0.14	0.28	0.04
PM_{10}						
Yes	331	1.05 (0.88, 1.26)	1.07 (0.88, 1.28)	1.03 (0.86, 1.24)	1.04 (0.86, 1.27)	1.10 (0.84, 1.43)
No	206	1.27 (1.01, 1.59)	1.25 (1.01, 1.55)	1.26 (0.98, 1.61)	1.21 (0.95, 1.53)	1.60 (1.15, 2.23)
p-value[c]		0.20	0.27	0.19	0.36	0.03
Current Regular Aspirin Use[b]						
$PM_{2.5}$						
Yes	321	0.92 (0.78, 1.10)	0.93 (0.79, 1.10)	0.88 (0.74, 1.06)	0.92 (0.77, 1.10)	0.86 (0.67, 1.09)
No	216	1.15 (0.94, 1.42)	1.06 (0.87, 1.29)	1.06 (0.87, 1.31)	1.15 (0.96, 1.38)	1.20 (0.92, 1.56)
p-value[c]		0.11	0.43	0.16	0.04	0.02
PM_{10}						
Yes	321	1.07 (0.89, 1.28)	1.11 (0.92, 1.33)	1.05 (0.87, 1.28)	0.98 (0.81, 1.19)	1.11 (0.85, 1.45)
No	216	1.24 (0.99, 1.56)	1.19 (0.96, 1.48)	1.19 (0.95, 1.48)	1.37 (1.06, 1.76)	1.56 (1.12, 2.17)
p-value[c]		0.32	0.60	0.39	0.04	0.04

[a]ORs calculated from conditional logistic regression models and presented by change in PM IQR ($PM_{2.5IQR}$ =8.73; PM_{10IQR} = 14.58). All ORs control for mean daily temperature [b] Self-report from biennial questionnaire :("Have you had a physician tell you that you have high blood pressure?", "Have you had a physician tell you that you have high cholesterol?", "Do you regularly take aspirin?" ("Yes" defined as 2+ tablets /week) [c] p-value from chi-sq tests of model heterogeneity

Supplemental Table 1. Odds Ratios and 95% Confidence Intervals of the Association between Short-term Particulate Matter Exposure and Ischemic Stroke by Season[a] in the Health Professionals Follow-up Study

	N	Lag 0 OR (95% CI)[b]	Lag 1 OR (95% CI)[b]	Lag 2 OR (95% CI)[b]	Lag 3 OR (95% CI)[b]	Lag0-3 (Avg) OR (95% CI)[b]
$PM_{2.5}$						
Winter	213	1.02 (0.81, 1.28)	1.00 (0.81, 1.24)	0.94 (0.73, 1.20)	0.96 (0.77, 1.18)	0.96 (0.71, 1.29)
Spring	184	0.91 (0.64, 1.31)	0.72 (0.50, 1.05)	0.90 (0.64, 1.26)	0.89 (0.62, 1.27)	0.75 (0.46, 1.22)
Summer	144	1.07 (0.80, 1.43)	1.08 (0.82, 1.43)	0.85 (0.62, 1.16)	1.15 (0.82, 1.61)	1.06 (0.70, 1.58)
Fall	175	0.98 (0.76, 1.26)	1.04 (0.81, 1.34)	1.07 (0.84, 1.36)	1.11 (0.87, 1.41)	1.10 (0.79, 1.52)
p-value[c]		0.91	0.35	0.69	0.60	0.62
PM_{10}						
Winter	213	1.13 (0.88, 1.46)	1.11 (0.87, 1.41)	1.06 (0.82, 1.36)	1.04 (0.79, 1.36)	1.16 (0.80, 1.69)
Spring	184	1.05 (0.81, 1.36)	0.89 (0.68, 1.15)	1.05 (0.81, 1.35)	1.26 (0.97, 1.64)	1.11 (0.77, 1.61)
Summer	144	0.97 (0.74, 1.28)	1.01 (0.75, 1.37)	0.88 (0.64, 1.20)	1.00 (0.73, 1.38)	0.93 (0.60, 1.44)
Fall	175	1.13 (0.88, 1.46)	1.22 (0.96, 1.55)	1.19 (0.93, 1.52)	1.10 (0.87, 1.40)	1.33 (0.95, 1.86)
p-value[c]		0.841	0.350	0.519	0.681	0.646

[a]Season defined by month of the year: Winter (Dec-Feb), Spring (Mar-May), Summer (Jun-Aug), Fall (Sep-Nov)

[b]ORs calculated from conditional logistic regression models stratified by season and presented by change in PM IQR ($PM_{2.5IQR}$ =8.73; PM_{10IQR} = 14.58). All ORs control for mean daily temperature

[c] p-value from chi-sq tests of model heterogeneity

Supplemental Table 2a. Odds Ratios and 95% Confidence Intervals of the Association between Short-term Exposure to PM2.5 and PM10 (Lag 0 to Lag 3) and Stroke by Method of Adjustment for Temperature[a] in the Health Professionals Follow-up Study

	N	Lag 0 OR (95% CI)[b]	Lag 1 OR (95% CI)[b]	Lag 2 OR (95% CI)[b]	Lag 3 OR (95% CI)[b]	Lag0_3 (Avg) OR (95% CI)[b]
$PM_{2.5}$	716					
Unadjusted		1.04 (0.93, 1.16)	0.97 (0.87, 1.08)	0.95 (0.85, 1.06)	1.00 (0.90, 1.12)	0.98 (0.84, 1.14)
Adj by Linear Term		1.01 (0.90, 1.13)	0.93 (0.83, 1.05)	0.93 (0.83, 1.05)	1.01 (0.90, 1.13)	0.95 (0.81, 1.11)
Adj by Spline		1.00 (0.88, 1.12)	0.92 (0.82, 1.04)	0.93 (0.82, 1.04)	1.01 (0.90, 1.13)	0.93 (0.79, 1.10)
PM_{10}	716					
Unadjusted		1.11 (0.99, 1.24)	1.09 (0.97, 1.22)	1.07 (0.95, 1.21)	1.08 (0.95, 1.22)	1.17 (0.99, 1.38)
Adj by Linear Term		1.08 (0.95, 1.23)	1.05 (0.93, 1.19)	1.06 (0.93, 1.20)	1.10 (0.96, 1.25)	1.15 (0.96, 1.38)
Adj by Spline		1.07 (0.94, 1.22)	1.05 (0.92, 1.19)	1.06 (0.93, 1.21)	1.10 (0.96, 1.26)	1.15 (0.95, 1.39)

[a]Differing methods for adjustment by mean daily temperature included: unadjusted, adjustment using a linear term, and adjustment using restricted cubic splines
[b]ORs calculated from conditional logistic regression models stratified by season and presented by change in PM IQR ($PM_{2.5IQR}$ =8.73; PM_{10IQR} = 14.58)

Supplemental Table 2b. Odds Ratios and 95% Confidence Intervals of the Association between Short-term Exposure to PM2.5 and PM10 (Lag 4 to Lag 6) and Stroke by Method of Adjustment for Temperature[a] in the Health Professionals Follow-up Study

	N	Lag 4 OR (95% CI)[b]	Lag 5 OR (95% CI)[b]	Lag 6 OR (95% CI)[b]
$PM_{2.5}$	716			
Unadjusted		0.97 (0.87, 1.09)	0.98 (0.88, 1.10)	0.98 (0.88, 1.10)
Adj by Linear Term		0.98 (0.88, 1.10)	1.00 (0.89, 1.12)	0.99 (0.88, 1.12)
Adj by Spline		0.99 (0.88, 1.11)	1.02 (0.91, 1.15)	1.00 (0.88, 1.13)
PM_{10}	716			
Unadjusted		1.01 (0.90, 1.15)	0.99 (0.87, 1.12)	0.96 (0.85, 1.09)
Adj by Linear Term		1.04 (0.91, 1.18)	1.02 (0.89, 1.16)	0.97 (0.85, 1.11)
Adj by Spline		1.04 (0.91, 1.19)	1.05 (0.91, 1.21)	0.98 (0.85, 1.12)

[a]Differing methods for adjustment by mean daily temperature included: unadjusted, adjustment using a linear term, and adjustment using restricted cubic splines
[b]ORs calculated from conditional logistic regression models stratified by season and presented by change in PM IQR ($PM_{2.5IQR}$ =8.73; PM_{10IQR} = 14.58)

Chapter 4: Associations between Short-term Ambient Particulate Matter Exposure and Stroke Hospitalizations in Maryland

Title page

Associations between Short-term Ambient Particulate Matter Exposure and Stroke Hospitalizations in Maryland

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Abstract:

Background: Stroke is the fifth leading cause of death and a major contributor to long-term disability in the United States. Many prior studies between exposure to short-term PM and stroke have examined risk by composite stroke outcomes. Additional studies are needed that examine both ischemic and hemorrhagic stroke separately, as the two stroke types potentially differ in the pathophysiology relevant to air pollution exposure.

Methods: In this study, we performed a time-stratified case-crossover analysis to examine the association between daily ambient $PM_{2.5}$ and total cerebrovascular, ischemic, and hemorrhagic stroke hospitalizations among adults in Maryland from 2003 to 2011. Conditional logistic regression models were used to obtain estimates of odds ratios and 95% confidence intervals associated with an interquartile range increase in $PM_{2.5}$ from 0 to 3 lag days.

Results: Overall, the OR between $PM_{2.5}$ and cerebrovascular hospitalization was 1.003 (95% CI: 0.996 – 1.011) per IQR increase of $PM_{2.5}$ on the day of hospitalization. In models stratified by stroke subtype, no association was found between $PM_{2.5}$ and either ischemic stroke hospitalizations (OR=1.007; 95% CI: 0.997 – 1.017) or hemorrhagic stroke hospitalizations (OR=1.004; 95% CI: 0.977 – 1.031).

Discussion: We found no evidence of an association between $PM_{2.5}$ exposure and cerebrovascular hospitalizations or the subset of ischemic or hemorrhagic stroke

hospitalizations in Maryland during the time period. Future studies should continue to examine risk by stroke subtype and examine potential modifiers of effect.

Target Journals: 1) Environmental Health 2) Science of the Total Environment 3) Environmental Research

Keywords: Particulate matter, stroke, air pollution, case-crossover

Introduction

Stroke is the fifth leading cause of death and a major contributor to long-term disability in the United States, with 795,000 people experiencing a new or recurrent stroke every year (Benjamin et al. 2017; CDC 2016b). While associations between short-term exposure to ambient air pollution and cardiovascular mortality and morbidity have been established among a large and growing body of research (Brook et al. 2010), the evidence linking particulate matter (PM) air pollution exposures to the risk of stroke remains equivocal. Prior studies of short-term PM exposure and stroke have provided inconsistent results, with some studies (Chan et al. 2006; Delfino et al. 2009a; Dominici et al. 2006) but not others (Alessandrini et al. 2013; Jalaludin et al. 2006; Lippmann et al. 2000; Lisabeth et al. 2008; Mechtouff et al. 2012; O'Donnell et al. 2011; Villeneuve et al. 2006) finding evidence of increased risk. Additional studies are needed to clarify if ambient particulate matter exposure is a modifiable risk factor for stroke.

Many prior studies of short-term pollutant-related stroke risk have examined risk by composite stroke outcomes, such as the combination of all cerebrovascular hospitalizations or deaths. However, both ischemic and hemorrhagic stroke are known to differ in their etiologies, outcomes, and potentially in the pathophysiology relevant to air pollution exposure (Ljungman and Mittleman 2014). For this reason, a recent review has called for additional studies classifying cerebrovascular events by stroke subtype (Wang et al. 2014). In addition, relatively few prior studies have been able to examine if risk for PM-related stroke differs by population subgroups.

Demographic characteristics such as age, gender, and race/ethnicity are well-established risk factors for stroke and may modify the association between PM and stroke via physiology, exposure patterns, activity patterns, or other mechanisms (Bell et al. 2013; Benjamin et al. 2017). While a few studies have examined these relationships (Franklin et al. 2006; Oudin et al. 2010; Villeneuve et al. 2006), others are needed. Such findings are of particular interest as they may identify populations most vulnerable to the effects of PM-related stroke, which may better inform effective public health prevention strategies.

In this study, we performed a time-stratified case-crossover analysis to examine the association between daily ambient fine particle concentrations (particles with diameter $\leq$2.5 μm, $PM_{2.5}$) and total cerebrovascular, ischemic, and hemorrhagic stroke hospitalizations among adults in Maryland from 2003 to 2011. We also investigated whether season and demographic characteristics (age, gender, and race/ethnicity) modified the relationship between PM exposure and ischemic or hemorrhagic stroke.

Methods

Hospital discharge data were obtained from the Maryland Department of Health and Mental Hygiene (DHMH). Hospital discharge data were obtained from all 48 acute care hospitals in Maryland. All acute care hospitalization records with a principal discharge diagnosis of cerebrovascular disease (International Classification of Diseases, Ninth Revision [ICD-9] code 430-438) with an admission date from January 1, 2003 to December 31, 2011 were included. Stroke subtypes were assigned

to patients based on ICD-9 code classifications (Kokotailo & Hill, 2005). ICD-9 codes 430 (subarachnoid hemorrhage), 431 (intracerebral hemorrhage), and 432 (other and unspecified intracranial hemorrhage) were classified as hemorrhagic stroke. ICD-9 codes 433 (occlusion and stenosis of precerebral arteries), 434 (occlusion of cerebral arteries), and 436 (acute but ill-defined cerebrovascular disease) were designated as ischemic stroke. ICD-9 codes 435 (TIA), 437 (other and ill-defined cerebrovascular disease), and 438 (late effects of cerebrovascular disease) were designated as other stroke types. Data elements for each record included county of residence, age, gender, race/ethnicity, and admission date. Approval for the usage of this information was obtained by institutional review boards at the University of Maryland and the Maryland DHMH.

Data for fine particulate matter ($PM_{2.5}$) was sourced from the CDC Wide-ranging Online Data for Epidemiologic Research (WONDER) online database (CDC 2016a). The pollution data were created from a modified regional surfacing algorithm based on prior work by Al-Hamdan et al. 2009 (Al-Hamdan et al. 2009). The algorithm uses collected monitor data from the U.S. Environmental Protection Agency (EPA) Air Quality System and remotely sensed aerosol optical depth from the National Aeronautics and Space Administration (NASA) Moderate Resolution Imaging Spectroradiometer (MODIS) to generate continuous spatial grids of daily $PM_{2.5}$ for the whole conterminous United States. County-level data were obtained by averaging values from all 10-km grids that had a centroid located within the subject county.

Daily maximum temperature values were obtained from the National Climatic Data Center (NCDC 2017). County-specific maximum temperature values were calculated for each calendar day from 2003 to 2011 by averaging all values from weather stations within the county. If no station data were available for a particular day, values from stations that were located within a 30 km radius of the county boundary were used.

We used a time-stratified case-crossover design to assess the association between $PM_{2.5}$ and stroke hospitalizations. Proposed by Maclure, the case-crossover design has increasingly been used to examine the effects of transient exposures on the risk of acute events (Maclure 1991). With this design, each case's exposure just prior to the event is compared to exposure at other referent periods. In this way, each case serves as his/her own control, and confounding by invariant and slowly changing risk factors is controlled. In order to prevent time-trend bias and to ensure unbiased conditional logistic regression estimates, we used a time-stratified approach for referent period selection (Janes et al. 2005). Month-long strata were used, and exposures during the case period (day of or days previous to stroke event) were compared to exposures on the same day of the week and in the same calendar month as the case period.

Using SAS 9.4 (SAS Institute Inc., Cary, NC), conditional logistic regression models were used to obtain estimates of odds ratios (ORs) and 95% confidence intervals (CIs) associated with an interquartile range (IQR) increase in $PM_{2.5}$. PM exposures on the day of the stroke event (lag 0), as well as exposures from 1 to 3 days previous to the stroke event (lag 1 to lag 3) were considered in separate models. Restricted cubic

splines were used to control for temperature, a potential confounder in this analysis. Effect modification of the relationship between PM and ischemic and hemorrhagic stroke was examined by season (warm: stroke occurring May-September, cold: stroke in October-April), age categories (18-69, 70 and older), gender and race/ethnicity (non-Hispanic black, non-Hispanic white) through stratified conditional logistic models. A sensitivity analysis also considered possible effect modification by four meteorological seasons defined as winter (December to February), spring (March to May), summer (June to August), and fall (September to November). Significance testing for effect modification was assessed using chi-square tests of model heterogeneity between the stratified effect estimates.

Results

In total, there were records of 163,057 cerebrovascular hospitalizations in Maryland from 2003 to 2011. Means and distributions of daily county-level $PM_{2.5}$ concentrations and maximum daily temperatures on the day of hospitalization are presented in Table 1. The mean on the day of hospitalization was 13.6μg/m^3, and 90% of the total values of $PM_{2.5}$ were between 5.2 and 26.6 μg/m^3.

The numbers of total, ischemic, and hemorrhagic stroke hospitalizations are presented by warm/cold season, age category, race/ethnicity, and gender in Table 2. Of the total 163,057 cerebrovascular hospitalizations, 95,865 (60.3%) were classified by primary ICD-9 diagnosis as ischemic strokes, 17,038 (8.2%) were classified as hemorrhagic strokes, and 50,154 (31.5%) were of other stroke subtypes. More hospitalizations took place in the seven months classified as having colder weather (58.1%) than in

the five months of warmer weather (41.9%). Relative percentages of the number of hospitalizations by warm or cold season did not differ by ischemic or hemorrhagic stroke subtype.

However, the number of hospitalizations and percentages among each age category did differ by stroke subtype. More ischemic stroke hospitalizations occurred among those 70 years or older (56.6%) compared to those ages 18 to 69 (43.5%). The opposite was true for hemorrhagic stroke hospitalizations; more occurred among those ages 18 to 69 (50.4%) than among those 70 years or older (49.7%). Reflective of total population distribution of Maryland, the majority of hospitalizations were among Non-Hispanic Whites (61.6%) compared to Non-Hispanic Blacks (30.7%), Hispanics (1.2%), or other or unknown race ethnicities (6.5%). Additionally, more females than males were hospitalized with both ischemic stroke (53.1% female to 46.9% male) and hemorrhagic stroke (53.9% female to 46.1% male).

ORs and 95% CIs for exposures to $PM_{2.5}$ and total stroke and by stroke subtype in Maryland are presented in Table 3. Overall, the OR between $PM_{2.5}$ and cerebrovascular hospitalization was 1.003 (95% CI: 0.996 – 1.011) per IQR increase of $PM_{2.5}$ on the day of hospitalization. In models stratified by stroke subtype, no association was found between $PM_{2.5}$ and either ischemic stroke hospitalizations (OR=1.007; 95% CI: 0.997 – 1.017) or hemorrhagic stroke hospitalizations (OR=1.004; 95% CI: 0.977 – 1.031). Similarly, no association was found among the other stroke subtypes and $PM_{2.5}$ exposure (OR=0.997; 95% CI: 0.984 – 1.011). Null

findings were consistent across all lag days for associations between $PM_{2.5}$ exposure and hospitalizations by stroke subtype.

Associations between $PM_{2.5}$ exposures and ischemic and hemorrhagic stroke hospitalizations stratified by warm and cold season are presented in Table 4. For ischemic stroke hospitalizations, the ORs for an IQR increase in $PM_{2.5}$ were 1.006 (95% CI: 0.992 – 1.020) during the cold season and 1.008 (95% CI: 0.994 – 1.023) during the warm season. For hemorrhagic stroke hospitalizations, the lag 0 ORs were 1.002 (95% CI: 0.966 – 1.040) during the cold season and 1.015 (95% CI: 0.975 – 1.058) during the warm season. No significant associations were found for other lag periods. Additionally, in tests of effect modification between stratified estimates, no significant associations were found between cold and warm season ORs for ischemic or hemorrhagic stroke ($p>0.05$).

Associations between $PM_{2.5}$ exposures and ischemic and hemorrhagic stroke hospitalizations stratified by age category, gender and race/ethnicity are presented in Table 5. No significant associations were found by any category for either ischemic or hemorrhagic stroke. For hemorrhagic stroke, lag day 0 ORs were slightly elevated for females (OR=1.021; 95% CI: 0.985 – 1.059) and those 70 years or older (OR=1.026; 95% CI: 0.987 – 1.067) compared to ORs for males (OR=0.984; 95% CI: 0.946 – 1.023) and those 18 to 69 (OR=0.984; 95% CI: 0.949 – 1.021). However, these differences were not significant in tests of heterogeneity. In the sensitivity analysis of associations between $PM_{2.5}$ and ischemic and hemorrhagic stroke by

meteorological season, no differences were found between stratified estimates in tests of heterogeneity (Supplemental Table 1).

Discussion

We found no evidence of an association between $PM_{2.5}$ exposure and cerebrovascular hospitalizations or the subset of ischemic or hemorrhagic stroke hospitalizations in Maryland from 2003 to 2011. Results remained null in stratified analyses by stroke subtype, season, age category, race/ethnicity, and gender. Results were consistent across lag periods, and no significant differences were found between stratified estimates in tests of heterogeneity.

Our findings of no relationship between short-term exposure to $PM_{2.5}$ and stroke hospitalizations are consistent with several previous studies (Alessandrini et al. 2013; Jalaludin et al. 2006; Lippmann et al. 2000; Lisabeth et al. 2008; Mechtouff et al. 2012; O'Donnell et al. 2011; Villeneuve et al. 2006). Similar to our study, the majority of these have used administrative databases to classify stroke or cerebrovascular hospitalizations and have used either a case-crossover or time-series design for analysis. However, a few studies have found positive significant results between total cerebrovascular hospitalizations and $PM_{2.5}$. The largest of these studies was Dominici et al., which evaluated the relationship among 11.5 million Medicare beneficiaries 65 years or older in 204 U.S. urban counties. They found a 0.8% increase (95% CI 0.3 – 1.3) in risk for cerebrovascular hospitalizations per 10ug/m3 increase in $PM_{2.5}$ (Dominici et al. 2006). Similarly, in a study of 10,330

cerebrovascular hospitalizations in California, Delfino et al. found a risk ratio of 1.019 (95% CI: 1.004 – 1.035) per 10μg/m^3 in 2-day moving average of $PM_{2.5}$ (Delfino et al. 2009a).

Several studies have used registries, validated medical records, or specific ICD-9 codes to examine the risk for ischemic and hemorrhagic stroke hospitalizations (Chan et al. 2006; Lisabeth et al. 2008; O'Donnell et al. 2011; Villeneuve et al. 2006; Wellenius GA et al. 2012). Studies examining ischemic and hemorrhagic stroke separately are needed as air pollution may affect underlying pathophysiological pathways differently (Ljungman and Mittleman 2014). Additionally, studies using validated stroke data may better avoid several biases related to the misclassification of the timing and coding of the stroke event (Lokken et al. 2009; Wang et al. 2014). In one study of 1,705 validated ischemic stroke patients in Boston, Wellenius et al. found a positive and significant relationship with short-term exposure to $PM_{2.5}$ (OR=1.11; 95% CI: 1.03 – 1.20 per IQR increase [6.4μg/m^3]) (Wellenius GA et al. 2012). However, two other studies using medically-reviewed cases found no significant relationship (Lisabeth et al. 2008; O'Donnell et al. 2011). Similar to our study, both Chan et al. and Villeneuve et al. separated ischemic strokes by ICD codes. Each study found no significant relationship between ischemic stroke and $PM_{2.5}$ (Chan et al. 2006; Villeneuve et al. 2006).

There are only a few previous studies which have examined short-term associations between $PM_{2.5}$ and hemorrhagic stroke, and all classified hemorrhagic stroke hospitalizations based on ICD codes (Chan et al. 2006; Villeneuve et al. 2006;

Yorifuji et al. 2011). Similar to our study, two studies reported null findings (Chan et al. 2006; Villeneuve et al. 2006). In the first, a study of 690 hemorrhagic stroke hospitalizations in Taiwan, Chan et al. found no relationship between $PM_{2.5}$ and hemorrhagic stroke hospitalizations (OR_{lag0}=0.870; 95% CI: 0.740 – 1.01 per IQR increase [19.73µg/m^3]) (Chan et al. 2006). Findings were consistently null across all lag periods. In the second, Villeneuve found null results for hemorrhagic stroke (OR_{lag0}=0.99; 95% CI: 0.90 – 1.08 per $PM_{2.5}$ IQR [6.3µg/m^3]) in their study of 2,329 stroke hospitalizations in Edmonton Canada (Villeneuve et al. 2006). However, a third study of 4,983 subarachnoid hemorrhage and 11,829 intracerebral hemorrhage fatal stroke events found significantly positive effects between subarachnoid hemorrhage and short-term exposure to $PM_{2.5}$ (Yorifuji et al. 2011). On the day before hospitalization, a 10µg/m^3 increase in $PM_{2.5}$ increased the risk of subarachnoid hemorrhage by 4.1% (95% CI: 1.1 – 7.2%) (Yorifuji et al. 2011).

Though we did not find differences in effects by season, the relationship between short-term PM exposure and stroke may differ by time of the year, depending on location (Jalaludin et al. 2006; Talbott et al. 2014; Tsai et al. 2003). In a study of over one million cerebrovascular hospitalizations in seven U.S. states, Talbott et al. found no consistent evidence of association between $PM_{2.5}$ and stroke hospitalizations when examined across the entire year (Talbott et al. 2014). However, there was evidence of seasonal differences, and those differences varied by location. For example, associations were significantly positive in Massachusetts during the cooler months (OR_{lag0}=1.045; 95% CI: 1.025 – 1.066) compared to warmer months (OR_{lag0}=0.885; 95% CI: 0.863 – 0.906). However, in Florida, ORs were higher and significantly

positive in the warmer months (OR_{lag0}=1.036; 95% CI: 1.021 – 1.052) and negative in the cooler months (OR_{lag0}=0.935; 95% CI: 0.920 – 0.950) (Talbott et al. 2014). Additional studies suggest that region may influence whether season modifies the effect between $PM_{2.5}$ and stroke. While a study in Sydney, Australia found reduced associations in warmer periods (Jalaludin et al. 2006), a study in Taiwan found elevated associations for ischemic stroke hospitalizations in days above 20 °C (Tsai et al. 2003). Not all studies have shown seasonal differences in the effect of $PM_{2.5.}$ Similar to our study, Villeneuve et al. found no differences by season for associations with $PM_{2.5}$ by ischemic or hemorrhagic stroke (Villeneuve et al. 2006). Whether differences by season in certain locations are due to the relationship with temperature, seasonal differences in PM composition, activity patterns that affect exposure, or another mechanism is not clear (Jalaludin et al. 2006; Talbott et al. 2014).

We found no differences in effect estimates for ischemic or hemorrhagic stroke by age category, gender, or race/ethnicity in this population. Studies examining effect modification are rather limited, but there are a few to which we can compare our results. Neither Delfino et al. nor Wellenius et al. found differences by age category in the association between stroke and $PM_{2.5}$ exposure (Delfino et al. 2009a; Wellenius GA et al. 2012). However, Franklin (2006) found a significantly higher risk of stroke mortality among those 75 or older compared to those younger than 75 years old ($p<0.05$) (Franklin et al. 2006). Our findings of no differences by gender are similar to those of Villeneuve and Oudin, but few others have examined this relationship (Oudin et al. 2010; Villeneuve et al. 2006). Likewise, we know of no other studies that have directly tested for effect modification by race/ethnicity in the relationship

between short-term exposure to $PM_{2.5}$ and ischemic and hemorrhagic stroke hospitalizations, specifically. However, in a study of PM_{10} and cerebrovascular mortality in 20 U.S. cities, Zeka et al. examined effect modification by race. They found no significant differences in effect estimates between blacks and whites (Zeka et al. 2006b). While there is limited evidence that the risk of stroke may be modified by these demographic characteristics, studies examining vulnerable population subgroups are important to better inform public health policy and develop effective prevention strategies.

This study has several notable strengths. The large number of cases in this study allowed for sufficient examination of risk within population subgroups, even for hemorrhagic stroke events, where previous analyses have often been under-powered. Another advantage of this study is the control of all time-invariant confounders through the case-crossover study design. Not only are descriptive characteristics like gender and age matched and controlled for, other factors such as obesity and hypertension are also controlled due to the relatively short windows between case and control periods. Additionally, we have selected a time-stratified design that controls for potential confounding by day of the week and seasonality.

There are several limitations to this study. Similar to most other studies of ambient air pollution and stroke, we were not able to assess individual-level exposures to PM. An individual's total exposure to PM represents the sum of their exposure to particles both of outdoor and indoor origin. Though studies have shown ambient concentrations of PM correlate relatively well with personal exposures (Avery et al.

2010), some exposure misclassification is unavoidable. Additionally, the use of county-level pollution estimates may bias estimates to the null in comparison to estimates at individual locations (Zeger et al. 2000). However, the use of modeled data from EPA for daily exposure estimates may represent an improvement over other studies reliant on a nearest monitor approach. As monitor locations are most often clustered around major cities, the use of modeled data allows for the inclusion of additional participants in rural areas.

There are also several limitations regarding the use of hospitalizations for this study. Several studies have identified that coding of stroke cases and subtypes by ICD-9 codes is not always reliable, as they depend both on the expertise of the clerical staff and the accuracy of the medical records (Kokotailo and Hill 2005; Reker et al. 2002). We were not able to separate first stroke hospitalizations from recurrent hospitalizations in this study. This would be problematic if a patient had multiple stroke events in a short time period, as reference or control periods at times before and after the event would no longer be appropriate. Another limitation of hospitalization data is the potential misclassification in exposure that occurs due to the time differential between stroke onset and hospitalization. This form of misclassification has been shown to bias effect estimates towards the null (Lokken et al. 2009).

Conclusion

We found no evidence of an association between $PM_{2.5}$ exposure and cerebrovascular hospitalizations or the subset of ischemic or hemorrhagic stroke hospitalizations in

Maryland during the time period (2003 to 2011). Future studies should continue to examine risk by stroke subtype and examine potential modifiers of effect. Examining potential effect modifiers was of major interest, as identifying those most vulnerable to the effects of ambient particulate exposures may inform future public health prevention policies. As the US EPA re-evaluates regulations on particulate matter every five years, findings from this and similar studies may influence and contribute to future risk and policy assessment documents.

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Tables

Table 1. Means and distributions of $PM_{2.5}$ and maximum daily temperature values on the day of cerebrovascular hospitalization in Maryland 2003-2011.

		Quantile								
	Mean	1	5	10	25	50	75	90	95	99
$PM_{2.5}$ ($\mu g/m^3$)	13.6	3.5	5.2	6.2	8.8	12.3	16.9	22.7	26.6	34.9
Max Daily Temp (°C)	18.4	-3.3	1.1	3.9	10.0	19.4	27.2	30.9	32.4	35.0

Table 2. Distributions of cerebrovascular hospitalizations by season, demographic subgroups, and stroke subtype

	Total Cerebrovascular Hospitalizations (n=163,057)	Ischemic Stroke (n=95,865)	Hemorrhagic Stroke (n=17,038)
	N (%)	N (%)	N (%)
Season			
Cold (Oct-Apr)	94726 (58.1%)	55676 (58.1%)	10263 (60.2%)
Warm (May-Sep)	68331 (41.9%)	40189 (41.9%)	6775 (39.8%)
Age Category			
18-69	75296 (46.2 %)	41658 (43.5 %)	8578 (50.4 %)
70+	87761 (53.8 %)	54207 (56.6 %)	8460 (49.7 %)
Race/Ethnicity			
NH Black	50054 (30.7%)	28087 (29.3%)	5608 (32.9%)
NH White	100478 (61.6%)	60366 (63.0%)	9612 (56.4%)
Hispanic	1979 (1.2%)	1061 (1.1%)	308 (1.8%)
Other or Unknown	10546 (6.5%)	6351 (6.6%)	1510 (8.9%)
Gender			
Male	73019 (44.8%)	44976 (46.9%)	8306 (48.8%)
Female	90038 (55.2%)	50889 (53.1%)	8732 (51.3%)

Hospitalizations classified by stroke subtype according to ICD-9 code: total cerebrovascular (430-438), ischemic stroke (433, 434, 436), hemorrhagic stroke (430-432).

Table 3: Odds ratios and 95% confidence intervals (CIs) for exposures to $PM_{2.5}$ and total stroke and by stroke subtype in Maryland, 2003-2011.

Hospitalization Type	Lag 0	Lag 1	Lag 2	Lag 3
All Cerebrovascular	1.003 (0.996, 1.011)	0.998 (0.991, 1.006)	0.995 (0.987, 1.003)	0.996 (0.989, 1.004)
Ischemic Stroke	1.007 (0.997, 1.017)	1.002 (0.992, 1.012)	0.992 (0.982, 1.002)	0.992 (0.983, 1.002)
Hemorrhagic Stroke	1.004 (0.977, 1.031)	0.981 (0.955, 1.008)	0.985 (0.959, 1.012)	0.978 (0.952, 1.005)
'Other' Stroke Subtype	0.997 (0.984, 1.011)	0.996 (0.983, 1.010)	1.005 (0.991, 1.019)	1.014 (1.000, 1.028)

ORs calculated from conditional logistic regression models and presented by change in $PM_{2.5}$ IQR (8.14μg/m^3). All models control for maximum daily temperature.

Table 4: Odds ratios and 95% confidence intervals (CIs) for exposures to $PM_{2.5}$ and ischemic or hemorrhagic stroke stratified by season in Maryland, 2003-2011.

Characteristic	Lag 0	Lag 1	Lag 2	Lag 3
Ischemic Stroke				
Cold (Oct-Apr)	1.006 (0.992, 1.020)	1.005 (0.991, 1.020)	0.990 (0.976, 1.004)	0.991 (0.977, 1.005)
Warm (May-Sep)	1.008 (0.994, 1.023)	1.001 (0.986, 1.015)	0.997 (0.983, 1.012)	0.998 (0.984, 1.013)
Hemorrhagic Stroke				
Cold (Oct-Apr)	1.002 (0.966, 1.040)	1.000 (0.963, 1.038)	0.994 (0.957, 1.031)	0.987 (0.951, 1.025)
Warm (May-Sep)	1.015 (0.975, 1.058)	0.963 (0.924, 1.004)	0.985 (0.945, 1.026)	0.966 (0.927, 1.007)

ORs calculated from conditional logistic regression models and presented by change in $PM_{2.5}$ IQR (8.14μg/m^3). All models control for maximum daily temperature.

Table 5: Odds ratios and 95% confidence intervals (CIs) for exposures to $PM_{2.5}$ and ischemic or hemorrhagic stroke stratified by demographic subgroup in Maryland, 2003-2011.

Characteristic	Lag 0	Lag 1	Lag 2	Lag 3
Ischemic Stroke				
Age 18-69	1.009 (0.994, 1.024)	0.999 (0.984, 1.014)	0.984 (0.970, 0.999)	0.996 (0.981, 1.011)
Age 70+	1.005 (0.992, 1.018)	1.005 (0.992, 1.018)	0.998 (0.985, 1.011)	0.990 (0.977, 1.003)
Male	1.013 (0.999, 1.028)	1.001 (0.987, 1.016)	0.994 (0.979, 1.008)	0.992 (0.978, 1.006)
Female	1.001 (0.988, 1.014)	1.003 (0.990, 1.017)	0.991 (0.978, 1.005)	0.993 (0.980, 1.006)
NH-Black	1.012 (0.994, 1.030)	1.011 (0.993, 1.029)	0.991 (0.973, 1.010)	0.992 (0.974, 1.010)
NH-White	1.005 (0.993, 1.018)	0.996 (0.983, 1.008)	0.992 (0.979, 1.004)	0.992 (0.980, 1.004)
Hemorrhagic Stroke				
Age 18-69	0.984 (0.949, 1.021)	0.964 (0.929, 1.001)	0.991 (0.955, 1.028)	0.979 (0.944, 1.016)
Age 70+	1.026 (0.987, 1.067)	1.001 (0.962, 1.042)	0.979 (0.941, 1.019)	0.977 (0.939, 1.016)
Male	0.984 (0.946, 1.023)	0.962 (0.924, 1.002)	0.979 (0.941, 1.019)	0.997 (0.958, 1.037)
Female	1.021 (0.985, 1.059)	0.997 (0.961, 1.035)	0.991 (0.955, 1.028)	0.962 (0.927, 0.998)
NH-Black	1.007 (0.962, 1.054)	0.999 (0.954, 1.046)	1.002 (0.957, 1.049)	1.005 (0.961, 1.052)
NH-White	0.999 (0.964, 1.035)	0.980 (0.946, 1.017)	0.990 (0.955, 1.027)	0.973 (0.938, 1.009)

ORs calculated from conditional logistic regression models and presented by change in $PM_{2.5}$ IQR ($8.14 \mu g/m^3$). All models control for maximum daily temperature.

Supplemental Table 1: Odds ratios and 95% confidence intervals (CIs) for exposures to $PM_{2.5}$ and ischemic or hemorrhagic stroke stratified by meteorological season in Maryland, 2003-2011.

Characteristic	Lag 0	Lag 1	Lag 2	Lag 3
Ischemic Stroke				
Winter	1.004 (0.984, 1.025)	1.014 (0.994, 1.035)	1.000 (0.980, 1.021)	1.008 (0.987, 1.029)
Spring	1.009 (0.986, 1.032)	1.005 (0.982, 1.029)	0.985 (0.962, 1.008)	0.998 (0.975, 1.022)
Summer	1.002 (0.985, 1.019)	0.993 (0.976, 1.010)	0.994 (0.977, 1.012)	0.993 (0.976, 1.011)
Fall	1.017 (0.996, 1.039)	1.002 (0.980, 1.024)	0.985 (0.964, 1.007)	0.975 (0.955, 0.996)
Hemorrhagic Stroke				
Winter	0.977 (0.925, 1.031)	0.960 (0.909, 1.015)	0.971 (0.919, 1.026)	0.985 (0.932, 1.041)
Spring	1.041 (0.978, 1.107)	0.996 (0.936, 1.060)	1.013 (0.952, 1.078)	1.032 (0.970, 1.097)
Summer	0.991 (0.944, 1.040)	0.954 (0.907, 1.003)	0.975 (0.928, 1.025)	0.941 (0.896, 0.989)
Fall	1.021 (0.963, 1.082)	1.026 (0.967, 1.088)	1.022 (0.963, 1.084)	0.980 (0.924, 1.039)

ORs calculated from conditional logistic regression models and presented by change in $PM_{2.5}$ IQR (8.14μg/m^3). All models control for maximum daily temperature.
Seasons defined by month of the year: Winter (Dec-Feb), Spring (Mar-May), Summer (Jun-Aug), Fall (Sep-Nov)

Chapter 5: Associations of Particulate Matter Exposures and Inflammatory Biomarkers in the Nurses' Health Study

Title Page

Associations of Particulate Matter Exposures and Inflammatory Biomarkers in the Nurses' Health Study

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Abstract

Background: Though a wide body of evidence suggests a link between cardiovascular outcomes and exposure to particulate matter (PM) air pollution, biological mechanisms for this association have not yet been fully explained. A clear understanding of these mechanisms is central to the development of better clinical treatment and prevention strategies.

Methods: Using controls from a series of nested case-control studies in the Nurses' Health Study (NHS) cohort, we examined associations between chronic exposure to $PM_{2.5}$, PM_{10}, and $PM_{2.5-10}$ assessed through spatiotemporal exposure models and four systemic and cardiovascular inflammatory markers: C-reactive protein (CRP), fibrinogen, intercellular adhesion molecule type-1 (ICAM-1), and interleukin-6 (IL-6). We also examined whether residential proximity to major roads was associated with inflammatory marker concentrations, and whether smoking acted as an effect modifier in these relationships.

Results: After adjusting for potential confounding variables, concentrations were elevated for both CRP (13.1%; 95% CI: 2.6 - 24.6) and IL-6 (8.4%; 95% CI: 1.7 – 15.6) among participants who lived close to a major roadway, but no significant results were found among models of 1- , 3-, or 12-month PM exposures. Smoking status did not act as an effect modifier in these associations.

Discussion: Though the fractions of particulate air pollution exposures we examined were not associated with the markers of inflammation chosen for this analysis, this

study provides additional evidence that proximity to traffic may influence cardiovascular-related inflammation. Future studies should continue to examine associations between systemic inflammation and exposures to particulate matter and other traffic-related air pollutants.

Target Journals: 1) Environmental Health Perspectives 2) Environmental Research

Keywords: Particulate matter, inflammatory biomarkers, CRP, IL-6, ICAM-1, fibrinogen, air pollution

Introduction

Though a wide body of evidence suggests a link between cardiovascular outcomes and exposure to particulate matter (PM) air pollution (Brook et al. 2010), biological mechanisms for this association have not yet been fully explained. A clear understanding of these mechanisms is central to the development of better clinical treatment and prevention strategies. Many mechanisms and pathways have been proposed including: oxidative stress, changes in autonomic function, and pulmonary inflammation leading to thrombosis and atherosclerosis (Utell et al. 2002). Though exact mechanisms remain unclear, many hypothesize an important role of inflammatory processes. Following inhalation, toxic substances present in PM (e.g., black carbon, metals) can cross epithelium of the airway and induce the production of proinflammatory cytokines and reactive oxygen species or alter the blood coagulation cascade (O'Toole et al. 2009; Uzoigwe et al. 2013). Such changes may lead to hypertensive responses, changes in autonomic cardiac control, atherosclerosis, as well as clot formation and movement; all of which may influence future ischemic and cardiovascular risk (Libby 2012; Loperena and Harrison 2017; Virdis et al. 2014). Establishing epidemiologic associations between air pollutants and blood-borne markers of inflammation can provide needed evidence to these potential mechanistic pathways and should be considered a research priority. Though there have been experimental and panel studies suggesting that short-term particulate matter exposures can induce inflammatory effects, fewer studies have been conducted on the role of sustained long-term exposures (Chen et al. 2015; Delfino et al. 2009b; Devlin et al. 2014; Dubowsky et al. 2006; Rückerl et al. 2006). Though a few recent studies

have been able to examine such associations (Hajat et al. 2015; Hampel et al. 2015; Hoffmann et al. 2009; Lanki et al. 2015), more studies with well-characterized information on potential confounding variables are needed.

The objective of this study was to determine the association between chronic exposure to particulate matter and systemic and cardiovascular inflammatory markers, including C-reactive protein (CRP), fibrinogen, intercellular adhesion molecule type-1 (ICAM-1), and interleukin-6 (IL-6) among controls from a series of nested case-control studies in the Nurses' Health Study (NHS) cohort. We used previously validated spatiotemporal exposure models of residential ambient PM to examine the effects of various exposure averaging times of $PM_{2.5}$ (PM with diameter $\leq$2.5 μm), PM_{10} (PM $\leq$10μm in diameter) and $PM_{2.5-10}$ (PM between 2.5 and 10 μm in diameter). We also examined whether residential proximity to major roads was associated with inflammatory marker concentrations, and whether smoking acted as an effect modifier in these relationships.

Methods

Study Population

The Nurses' Health Study cohort was initiated in 1976 and included female registered nurses 30 to 55 years old, who were residing in eleven states at the time of recruitment (NHS 2016). The ongoing prospective cohort consists of 121,700 participants that have been followed with biennial questionnaires for updates on

diseases, health status, and health-related conditions. The response rates are > 90% for each follow-up cycle.

Between 1989 and 1990, 32,826 participants (ages 43 to 70) who were free of diagnosed diabetes, coronary heart disease, stroke, or cancer provided blood samples using a mailed phlebotomy kit. Though blood samples were collected from many participants, serum assays were only conducted as part of individual nested case-control studies. Serum assays from healthy controls of four nested case-control studies (diabetes [N=760], myocardial infarction [N=486], breast cancer [N=1238], and macular degeneration [N=462]) were used for this study. Each of these studies tested for one or more of the four biomarkers of interest. Duplicate records (n=33) for controls that were in more than one nested case-control study were removed. Preference was given to the case-control study in which more assays were available. The study was approved by the Internal Review Board of Brigham and Women's Hospital.

Blood collection and biomarker measurement

Blood samples were transported from participants on ice overnight to the laboratory along with a completed questionnaire (included time of blood draw). The samples were then centrifuged, divided into aliquots and stored in the liquid nitrogen freezers. Ninety-seven percent of samples arrived within 26 h of phlebotomy (Hu et al. 2004). The concentrations of CRP and fibrinogen were measured with an immunoturbidimetric assay using reagents and calibrators from Denka Seiken

(Niigata, Japan). IL-6 and ICAM-1 were measured by ELISA assay (R & D Systems, Minneapolis, MN) that employs the quantitative sandwich enzyme immune assay technique. Studies on the sample collection in this population show overall within subject reliability of measures over time and limited effects of transport conditions (Hankinson et al. 1989; Pai et al. 2002).

Exposure Assessment

As part of the questionnaire mailing process, residential address information for NHS participants is updated every 2 years. Addresses were geocoded to obtain the corresponding latitude and longitude. Participants with missing an address or residing outside of the conterminous US were excluded from analysis (n=8). For women with a street segment–level geocode, distance to the nearest road (meters) was determined using geographic information system (GIS) software (ArcGIS, version 9.3; ESRI, Redlands, CA) and the ESRI Streetmap Pro2007 data set. Nearest distance was calculated as the shortest distance between the residential geocode and the following road classes as defined by the U.S. Census Bureau: A1 (primary roads, typically interstate highways, with limited access, division between the opposing directions of traffic, and defined exits), A2 (primary major, non-interstate highways and major roads without access restrictions), A3 (smaller, secondary roads, usually with more than two lanes) (U.S. Census Bureau 2001). Nearest distance was divided into three categories (0–49m, 50–199m, and $\geq$ 200 m) for analysis.

Ambient GIS-based spatiotemporal exposure model predictions of $PM_{2.5}$, PM_{10}, and $PM_{2.5\text{-}10}$ were assigned to each geocoded location for all months starting in

January 1988 for NHS participants living in the continental United States. Details of the models are covered in Yanosky et al, 2014 (Yanosky et al. 2014). Briefly, monthly mean $PM_{2.5}$ and PM_{10} values were calculated from several monitoring networks – primarily the U.S. EPA's Air Quality System (AQS) network, but also the Interagency Monitoring of Protected Visual Environments (IMPROVE), Southern Aerosol Research and Characterization Study (SEARCH), and others. Separate PM prediction surfaces for each month and each PM size fraction were created by using generalized additive mixed models with terms for time and monthly penalized spline smooth terms for a number of geospatial and meteorological covariates. Considered covariates included: distance to nearest road for U.S. Census road classes A1-A4, smoothed county-level population density, urban land use within 1 km, elevation, point-source emissions density within 7.5 km, smoothed monthly average wind speed, temperature, total precipitation, air stagnation and tract-level population density (for PM_{10}). By subtraction of the monthly PM_{10} and $PM_{2.5}$ estimates, information was also obtained on $PM_{2.5–10}$. Cross-validation results demonstrated that the models had high predictive accuracy (cross-validation R^2 values of 0.59 and 0.76 for PM_{10} and pre-1999 $PM_{2.5}$, respectively).

Covariates

Biannual follow-up questionnaires for the NHS cohort provide information on many risk factors and health-related behaviors. Potential confounding variables chosen for this analysis included variables matched by the individual nested case-control studies as well as other variables chosen *a priori* based on previous literature (Hajat et al.

2015; Ostro et al. 2014). Potential confounders matched by the nested case-control studies included: season (blood draw in Dec-Feb as winter, Mar-May as spring, Jun-Aug as summer, Sep-Nov as Fall) age (months), smoking status (never, past, current, missing), and use of postmenopausal hormones (nonuser, current user, premenopausal, unknown). Additional potential confounders included: BMI (kg/m^2) , pack-years smoked, physical activity (missing, <3, 3 to <18, ≥18 metabolic equivalent hours/week), hypertensive status (yes or no self-response to physician diagnosis), residential census tract median household income (based on 1990 census; (U.S. Census Bureau 1990)), alcohol consumption (missing, 0g/day, ≥0.1 g/day), MI family history (yes or no), and diet (measured by Alternate Healthy Eating Index (Chiuve et al. 2012)).

Statistical Analysis

To reduce excessive skewness of the distributions and to meet assumptions of the regression analyses, biomarker data were log-transformed and excessive outliers were excluded (CRP>10mg/l, fibrinogen>516 mg/dl, IL-6>7pg/ml, ICAM-1>424ng/ml). Geometric means were used to present the distributions of biomarkers by PM quartile. Multivariable linear regression models were used to test the hypothesis that PM exposures are associated with inflammatory biomarkers. Potential confounding variables were incorporated into the models in two stages. First crude models (basic) adjusted for variables which were matched in the individual nested case-control studies and included: age, season, menopausal status and use of post-menopausal hormones, smoking status, and fixed variables for the nested case-control study from

which the biomarker assay was obtained. Next, other established confounders and potential confounders were added to fully adjusted models; these include: BMI, median household income, pack-yrs smoked, alcohol use, physical activity, hypertensive status, MI family history, and diet. To examine the sensitivity of varying exposure averaging times, model sets were repeated for 1-, 3-, and 12-month exposures. Results from linear regression analysis of the PM fraction models are presented per interquartile range (IQR) in PM and as percent difference for each biomarker along with the corresponding 95% confidence interval. The presentation of linear regression effect estimates as percent difference was done to ease interpretation and provide consistency across multiple biomarkers. Since the biomarker values have been log-transformed, percent difference was calculated by exponentiating both sides of the linear regression equation, so that the exponentiated regression coefficient represents the relative change of the biomarker on the unlogged scale per IQR increase in PM. Basic and fully adjusted distance to road models included two exposure terms corresponding to nearest distance to an A1-A3 roadway (1-49m, and 50-199m). Thus, results from distance to road models are presented as percent difference in the biomarker concentration in comparison to concentrations among those living greater than 200m from an A1-A3 road. In order to test for effect modification by smoking, interaction terms were included in separate regression models.

Results

There were a total of 2946 participants with one or more assayed biomarker and complete exposure information used for this study. Descriptive characteristics of the total study population are presented in Table 1. During the period of blood collection, the average age of participants was 59 years, and ranged from 43 to 70 years of age. Most women were never (45.5%) or former smokers (40.0%). Also, most participants reported at least occasional alcohol use (78.6%, 0.1+ g/d) and modest levels of physical activity (78.6%, >3 MET hr/week). Roughly a third of study participants reported having a family history of MI (37.0%) or having had received a physician diagnosis of high blood pressure (30.2%).

Arithmetic means and standard deviations of the exposure variables and biomarker measurements are presented in Table 2. The 12-month means from the spatiotemporal models for PM exposures were 16.4, 27.9, and 11.5μg/m^3 for $PM_{2.5,}$ $PM_{10,}$ and $PM_{2.5-10}$, respectively. Means were relatively consistent across exposure averaging times, but standard deviations and interquartile ranges were larger for the 1- and 3-month averaging times. Approximately 45% of participants resided within 200m away from an A1 to A3 roadway, with 17.2% residing within 50 meters. The number of assays for each of the four biomarkers of interest varied as each nested case-control study assayed different biomarkers. In total, there were 2923 measurements for CRP, 1695 for ICAM-1, 1643 for IL-6, and 944 for fibrinogen.

Geometric means of each blood biomarker are presented by 12-month PM quartile and by distance to road category in Table 3. Means for fibrinogen and IL-6 did not vary consistently across PM quartiles. Mean concentrations of ICAM-1 decreased monotonically across $PM_{2.5}$ quartiles, though a finding of trend was not significant (p=0.058). Means for CRP were higher among the highest quartile of both PM_{10} and $PM_{2.5-10}$, but in tests of trend only the elevated findings within higher $PM_{2.5-10}$ quartiles reached statistical significance (p-trend=0.021). CRP concentrations were also elevated among those living closer to an A1-A3 road (1.79mg/L within 50 meters compared to 1.56mg/L further than 200m; p-trend=0.022). Mean concentrations of fibrinogen and IL-6 were also lowest among those living more than 200m from a roadway, though trend tests were not statistically significant.

Multivariable linear regression results showing percent differences in biomarker concentration by interquartile increase in PM exposure are presented in Table 4. After adjusting for confounders, no statistically significant results were found by any of the three PM fractions or any of the three exposure averaging times. Percent differences per IQR in PM were generally lower for $PM_{2.5}$ and higher for $PM_{2.5-10}$ for each of the assayed biomarkers. For the fully adjusted12-month models, results for CRP ranged from a -2.3% change (95% CI: -7.0 to 2.6%) per 7.85ug/m3 increase in $PM_{2.5}$ to a 2.1% change (95% CI: -1.6 to 5.8%) per 5.51$\mu g/m^3$ increase in $PM_{2.5-10}$. Also in fully adjusted models, results for IL-6 and ICAM-1 were consistently negative for all PM fractions and again were lower for $PM_{2.5}$ models in comparison to $PM_{2.5}$-10 models. For example, IL-6 models ranged from a -2.7% change (95% CI: -5.7 to 0.4%) per

7.26ug/m3 increase in 1-month $PM_{2.5}$ to a -0.2% change (95% CI: -3.0 to 2.7%) per 5.51ug/m3 increase in 12-month $PM_{2.5}$-10. Likewise, for ICAM-1 estimates ranged from a -0.6% change (95% CI: -1.8 to 0.6%) per 7.26ug/m3 increase in 1-month $PM_{2.5}$ to a 0.0% change (95% CI: -1.1 to 1.1%) per 5.89ug/m3 increase in 3-month $PM_{2.5\text{-}10}$. Estimates were positive, though not significant, for fibrinogen and increasing PM levels [range: 0.2% change (95% CI: -1.6 to 2.1%) per 7.85 12-month $PM_{2.5}$ to 0.9% change (95% CI: -0.6 to 2.3%) per 5.51 12-month $PM_{2.5}$-10].

In contrast to the PM models, we found significant associations for CRP and IL-6 in distance to road models (Table 5). Those living within 50m of a major roadway had 13.1% (95% CI: 2.6 to 24.6%) higher CRP concentrations compared to those living further than 200 meters from a roadway in fully adjusted models. In IL-6 distance to road models, higher concentrations were found among those living 50-199 meters from a major roadway compared to those living further than 200m (8.4% higher; 95% CI: 1.7 to 15.6%). Forest plots of both PM and distance to road models are presented by biomarker in Figures 1-4. In models including interaction terms by smoking status, no significant effect modification was found ($p>0.05$, data not shown).

Discussion

In this study, we found significant increases in CRP and IL-6 concentrations among participants who lived close to a major roadway, but not among models using estimates of ambient PM exposures from spatiotemporal statistical models. Findings

for fibrinogen and ICAM-1 were null for all exposure estimates. Though confidence intervals overlapped, estimates were higher among $PM_{2.5-10}$ models compared to $PM_{2.5}$ models. We noticed no differences by the tested exposure averaging times (1, 3, or 12 months) and found no evidence of effect modification by smoking status.

This study is one of few to examine the relationship between medium- and long-term exposures to PM and inflammatory markers in a cohort with well-characterized information on potential confounders. Hajat et al. (2015) examined 6,814 participants aged 45 to 84 in the Multi-Ethnic Study of Atherosclerosis (MESA) between 2002 and 2012 (Hajat et al. 2015). The authors examined several day-long exposures and a cumulative one year exposure to $PM_{2.5,}$ nitrogen oxides (NO_x), nitrogen dioxide (NO_2) and black carbon. In year-long $PM_{2.5}$ models, the authors found no differences by ICAM-1, fibrinogen or CRP, similar to our study, but found a significant increase in models of IL-6. After controlling for confounders, a 5$\mu g/m^3$ increase in yearly $PM_{2.5}$ exposures was associated with 6% higher IL-6 concentrations (95% CI = 2%, 9%). They also noticed this change was higher among smokers compared to nonsmokers, a difference not evident in our study. In addition to models using $PM_{2.5}$ estimates, the authors also examined biomarker differences by levels of NO_x, NO_2, and black carbon, all markers of traffic-related pollution (TRAP). Unlike our proximity to road findings for CRP and IL-6, they found no differences by the TRAP pollutants for CRP, IL-6, fibrinogen or ICAM-1(Hajat et al. 2015).

In another study of 4814 participants 45-75 years of age, from three large German cities, Hoffman et al examined changes in CRP and fibrinogen in response to annual $PM_{2.5}$ exposures and by distance to road categories (Hoffmann et al. 2009). Per IQR increase in $PM_{2.5}$ (3.91 $\mu g/m^3$), significant increases were seen for men but not women with both high-sensitivity CRP (hs-CRP) (men: 23.9% increase; 95% CI: 4.1 to 47.4; women: 1.0% decrease; 95% CI: -16.5 to 17.3) and fibrinogen (men: 3.9% increase; 95% CI: 0.3 to 7.7; Women: 1.5% increase; 95% CI: -1.9 to 5.1). Our results in the NHS cohort, a cohort of women with a similar age distribution, were similarly null for PM models. In contrast to our study, Hoffman et al. did not find differences in hs-CRP concentrations among men or women participants living within 50 or within 200m of a roadway.

In another European study and one of the largest studies to date, Lanki et al. used land-use regression models and proximity/intensity of traffic exposures to examine associations with 21,558 hs-CRP measurements and 17,428 fibrinogen measurements as part of the multi-cohort European Study of Cohorts for Air Pollution Effects (ESCAPE) project. As in our study, the authors examined associations by both fine and coarse PM fractions. In adjusted models, they found no significant associations with CRP or fibrinogen with $PM_{2.5,}$ PM_{10} or $PM_{2.5-10}$. However, cohort participants living on a busy (> 10,000 vehicles/day) road had elevated CRP values (10.2%; 95% CI: 2.4, 18.8%, compared with persons living on a residential street with < 1,000 vehicles/day). Though that study used a measure of traffic intensity at the nearest roadway, the magnitude of their finding is similar to ours (13.1% increase in CRP)

among those living near a major roadway. Another study of the ESCAPE multi-cohort examined associations among the same participants between CRP concentrations and land-use regression models by PM constituents. They found an increased and significant association between the $PM_{2.5}$ copper fraction and CRP (6.3%; 95% CI: 0.7 to 12.3%). Results from this study are important as they highlight that certain PM constituents, in this case, a primarily traffic-derived constituent, may be the primary drivers of PM-related inflammatory changes.

C-reactive protein is a protein produced by the liver and is considered one of the best measures of the acute phase response to an infectious disease, tissue damage, or inflammation (Gabay and Kushner 1999; Pope 2001). CRP has been shown in multiple epidemiologic studies to predict incident myocardial infarction, stroke, and sudden cardiac death (Ridker 2003), and it may also have a direct role in the development of atherosclerosis (Libby 2002). Though CRP has been shown to increase after short-term exposures in both epidemiological and experimental studies (Chen et al. 2015; Chuang et al. 2007; Delfino et al. 2009b; Devlin et al. 2014; Dubowsky et al. 2006; Strak et al. 2013). the few studies examining medium and long-term ambient PM exposures have been inconsistent (Hajat et al. 2015; Hoffmann et al. 2009; Ostro et al. 2014; Zeka et al. 2006a). Similarly, previous studies of associations between proximity to traffic and CRP have also been mixed. Our results of increased concentrations among those living close to a major roadway match closely with Lanke (2015) and Rioux (2010) but not with Hoffman et al. (Hoffmann et al. 2009; Lanki et al. 2015; Rioux et al. 2010).

In addition to the study by Hajat et al, two other studies have shown associations between IL-6 and NO_2, (a TRAP pollutant)(Chuang et al. 2011; Panasevich et al. 2009). Similar to our study, Jiang et al. also found elevated concentrations among participants living within 50 meters of a major road (Jiang et al. 2016). IL-6, a pro-inflammatory cytokine secreted by T cells and macrophages at the site of injury, has been shown in toxicological studies to be released in macrophages exposed to PM_{10} (Becker et al. 2005). Persistent inflammation in the lung may then invoke a general systemic inflammatory response as pro-inflammatory cytokines diffuse into systemic circulation (Tamagawa et al. 2008).

Both fibrinogen and ICAM-1 are glycoproteins important in their roles on the blood coagulation cascade and binding and migration of immune-associated molecules, respectively. Previous literature on a whole has also been consistent in their associations with cardiovascular risk factors (Blann et al. 1997; Chae et al. 2001; Rohde et al. 1999) and associations with cardiovascular diseases (Danesh et al. 1998; Davalos and Akassoglou 2012; Haim et al. 2002; Lind et al. 2001; Ridker et al. 1998). Though we found no evidence of in this study, others have found associations with elevated PM levels and fibrinogen (Chen et al. 2015; Chuang et al. 2007; Hildebrandt et al. 2009; Schwartz 2001) or ICAM-1(O'Neill et al. 2007).

Our findings of higher effect estimates among coarse PM models compared to fine PM models deserves further investigation. Though confidence intervals overlapped,

this finding was consistent in all but one fully adjusted model (1-month fibrinogen). In contrast to fine PM, the coarse size fraction is usually dominated by non-exhaust sources, such as road surface abrasions and brake and tire wear (Ketzel et al. 2007). In addition, dispersion distances vary between coarse and fine particles, as coarse particles remain suspended for shorter time periods and travel shorter distances from roadways. Thus, the relatively higher associations among the coarse fraction models may help to further interpret our findings of increased and significant relationships with roadway proximity for CRP and IL-6. Further investigation is needed to determine if the positive association with proximity to major roadway seen in our study is influenced by PM exposures of certain size fractions, specific constituents, or other gaseous pollutants associated with traffic. It is also possible that other factors may be responsible for increased cardiovascular effects or inflammation associated with proximity to roadways or traffic. Road noise, for example, has also been linked to adverse health outcomes, and the separation of effects from air pollution is difficult to separate (Fecht et al. 2016).

There are several notable strengths of this study. Few previous studies have looked at associations between PM exposures and biomarkers of inflammation in large cohorts with well-characterized information on potential confounders. Reliable information on potential confounding variables is critical in such studies as certain inflammatory biomarkers of interest can be associated with cardiovascular risk factors outside of clinical disease. Our use of robust and validated spatiotemporal statistical models may capture more spatial variability in exposure estimates than the use of estimates from

nearest or central monitoring locations. Additionally, the use of a nationwide cohort and exposure models extends the geographic scope of included subjects beyond studies limited to small regions or individual cities.

This study also has several limitations. Short-term PM (in terms of days) can also be an important predictor of inflammatory changes in addition to the medium- or long-term exposures examined in our study. With that said, two recent studies examined both short and long-term exposures simultaneously and found that long-term associations were not greatly impacted by the inclusion of short-terms exposures in the same models (Hajat et al. 2015; Hoffmann et al. 2009). Another study by Zeka et al. found that 1-month PM exposures were more strongly associated with CRP and fibrinogen than 2-day or 1-week exposures (Zeka et al. 2006a). Further investigation is needed to determine the extent to which short and long-term exposures conflate to influence cardiovascular risk and whether they act on different pathophysiologic mechanisms involving inflammation. Another limitation for this study was our inability to account for non-residential exposures or time-activity patterns that might influence total PM exposure. Though this is an important source of exposure misclassification, studies have shown overall that ambient concentrations of PM correlate relatively well with personal exposures (Avery et al. 2010). Generalizability of this cohort is also limited by the selection of healthy controls selected by the individual nested case-control studies as well as the limited ranges of age, race/ethnicity, and socioeconomic status of participants in the NHS.

Conclusion

The fractions of particulate air pollution exposures we examined were not associated with the markers of inflammation chosen for this analysis. However, after adjusting for potential confounding variables, concentrations of both CRP and IL-6 were elevated among participants living close to a major roadway compared to those living further away, providing some evidence that proximity to traffic may influence inflammatory markers of cardiovascular risk. Future studies should continue to examine associations between systemic inflammation and exposures to particulate matter and other traffic-related air pollutants. Studies examining mechanistic pathways are a crucial piece to establishing causal pathways between exposure and subsequent health outcomes. Such work provides a crucial 'how' to the studies of association between exposure and disease. The EPA incorporates such work in their integrated science assessment (ISA) documents that provide a comprehensive review, synthesis and evaluation of the most policy-relevant science. These documents directly inform future policy and air quality regulations.

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Tables

Table 1. Descriptive characteristics of 2946 NHS participants with one or more assayed biomarker measurement

Characteristics	mean (SD) or %
Age [years]	59.0 (7.7)
Census tract median household income ($)	65,448 (26,388)
BMI [kg/m^2]	25.6 (5.2)
Pack-years of smoking[a]	24.3 (20.4)
Smoking status (%)	
Current	14.1
Past	40.0
Never	45.5
Missing	0.4
Alcohol category (%)	
Non-drinker (0 g/day)	18.8
0.1+ g/d	78.6
Missing	2.6
Physical activity (%)	
<3 MET hr/week	20.6
3 to < 18 MET hr/week	46.7
18+ MET hr/week	31.9
Missing	0.8
Postmenopausal hormone use (%)	
Never or Past Use	48.4
Current use	35.3
Premenopausal	15.5
Unknown	0.8
MI Family History (%)	37.0
High Blood Pressure (%)	30.2

[a]Among current and former smokers only

Table 2. Arithmetic means and standard deviations or categorical distributions of exposure variables and biomarker concentrations

Exposure or Biomarker	N	Mean (SD)[a] or %
$PM_{2.5}$ ($\mu g/m^3$)		
1-Month	2946	16.6 (5.9)
3-Month	2946	16.5 (5.1)
12-Month	2946	16.4 (3.8)
PM_{10} ($\mu g/m^3$)		
1-Month	2946	27.3 (9.9)
3-Month	2946	27.5 (9.3)
12-Month	2946	27.9 (7.5)
$PM_{2.5-10}$ ($\mu g/m^3$)		
1-Month	2946	10.7 (6.2)
3-Month	2946	11.0 (6.1)
12-Month	2946	11.5 (5.7)
Distance to Road (meters)[b]		
≥200m	1622	55.0%
50-199m	818	27.7%
0-49m	506	17.2%
CRP (mg/L)	2923	3.1 (4.6)
IL-6 (mg/dl)	1643	2.3 (2.8)
Fibrinogen (pg/ml)	944	364.8 (88.3)
ICAM-1 (ng/ml)	1695	278.8 (90.4)

[a]Arithmetic means and standard deviations

[b] Included A1-A3 Categories as defined by U.S. Census Bureau, 2001. A1 (primary roads, typically interstate highways, with limited access, division between the opposing directions of traffic, and defined exits); A2 (primary major, non-interstate highways and major roads without access restrictions); A3 (smaller, secondary roads, usually with more than two lanes)

Table 3. Geometric means of inflammatory biomarkers and tests of trend by 12-month PM quartile or distance to road category

PM Quartile Or Distance Category	CRP (mg/L)	Fibrinogen (mg/dl)	IL-6 (pg/ml)	ICAM-1 (ng/ml)
$PM_{2.5}$ ($\mu g/m^3$)[a]				
4.78 – 13.86	1.74	349.69	1.60	273.56
13.86 – 16.42	1.42	362.35	1.58	268.20
16.42 – 19.11	1.58	357.90	1.68	267.88
19.11 – 30.76	1.71	346.82	1.61	263.47
p-trend[b]	0.803	0.605	0.647	0.058
PM_{10} ($\mu g/m^3$)[a]				
10.64 – 23.06	1.53	356.67	1.71	266.90
23.06 – 26.54	1.56	352.67	1.53	275.73
26.54 – 30.91	1.55	354.43	1.61	266.08
30.91 – 78.70	1.76	351.97	1.61	264.08
p-trend[b]	0.154	0.630	0.451	0.263
$PM_{2.5-10}$ ($\mu g/m^3$)[a]				
2.72 – 7.84	1.56	350.87	1.63	266.28
7.84 – 9.97	1.51	365.18	1.65	271.11
9.97 – 13.35	1.61	355.69	1.63	266.50
13.35 – 47.94	1.78	344.52	1.55	268.87
p-trend[b]	**0.021**	0.266	0.313	0.844
Distance to Road[c]				
>200 m	1.56	350.44	1.55	268.09
50-199 m	1.61	355.72	1.73	267.91
0-49 m	1.79	361.51	1.69	266.80
p-trend[b]	**0.022**	0.158	0.096	0.795

[a] PM exposures are 12-month annual average before blood draw

[b] p-value for test of trend

[c] Included A1-A3 Categories as defined by U.S. Census Bureau, 2001. A1 (primary roads, typically interstate highways, with limited access, division between the opposing directions of traffic, and defined exits); A2 (primary major, non-interstate highways and major roads without access restrictions); A3 (smaller, secondary roads, usually with more than two lanes)

Table 4. Percent differences and 95% confidence intervals of markers of inflammation per interquartile increase in PM exposure

	CRP		IL-6		Fibrinogen		ICAM-1	
	Basic-Adj[a]	Full-Adj[b]	Basic-Adj[a]	Full-Adj[b]	Basic-Adj[a]	Full-Adj[b]	Basic-Adj[a]	Full-Adj[b]
$PM_{2.5}$								
1 mo	0.5 (-3.9 to 5.0)	-0.2 (-4.2 to 3.9)	-2.4 (-5.5 to 0.8)	-2.7 (-5.7 to 0.4)	0.8 (-0.9 to 2.4)	0.8 (-0.9 to 2.4)	-0.6 (-1.7 to 0.6)	-0.6 (-1.8 to 0.6)
3 mo	-0.5 (-5.4 to 4.6)	-1.1 (-5.5 to 3.5)	-2.1 (-5.4 to 1.5)	-2.2 (-5.4 to 1.2)	0.5 (-1.2 to 2.2)	0.5 (-1.2 to 2.2)	-0.6 (-1.9 to 0.7)	-0.6 (-1.9 to 0.7)
12 mo	-1.2 (-6.4 to 4.3)	-2.3 (-7.0 to 2.6)	-1.7 (-5.5 to 2.2)	-2.4 (-6.0 to 1.3)	0.2 (-1.6 to 2.1)	0.2 (-1.6 to 2.1)	0.0 (-1.5 to 1.4)	-0.1 (-1.6 to 1.3)
PM_{10}								
1 mo	-0.5 (-3.1 to 2.3)	0.4 (-2.1 to 2.9)	-1.5 (-3.4 to 0.5)	-1.3 (-3.2 to 0.6)	0.5 (-0.5 to 1.6)	0.5 (-0.5 to 1.6)	-0.3 (-1.0 to 0.4)	-0.3 (-1.0 to 0.5)
3 mo	-0.9 (-3.6 to 1.9)	0.1 (-2.4 to 2.7)	-1.2 (-3.2 to 0.8)	-0.9 (-2.8 to 1.1)	0.5 (-0.5 to 1.5)	0.5 (-0.5 to 1.5)	-0.2 (-1.0 to 0.5)	-0.2 (-1.0 to 0.5)
12 mo	-0.7 (-3.5 to 2.1)	0.5 (-2.1 to 3.1)	-1.1 (-3.1 to 1.0)	-0.8 (-2.8 to 1.2)	0.5 (-0.5 to 1.5)	0.5 (-0.5 to 1.5)	-0.1 (-0.8 to 0.7)	-0.1 (-0.8 to 0.7)
$PM_{2.5-10}$								
1 mo	-1.5 (-5.4 to 2.5)	1.1 (-2.6 to 4.8)	-1.3 (-4.3 to 1.7)	-0.6 (-3.5 to 2.4)	0.6 (-0.9 to 2.2)	0.6 (-0.9 to 2.2)	-0.2 (-1.3 to 0.9)	-0.1 (-1.2 to 1.0)
3 mo	-1.5 (-5.3 to 2.4)	1.0 (-2.6 to 4.7)	-1.2 (-4.1 to 1.8)	-0.3 (-3.1 to 2.6)	0.8 (-0.7 to 2.3)	0.8 (-0.7 to 2.3)	-0.1 (-1.2 to 1.0)	0.0 (-1.1 to 1.1)
12 mo	-0.7 (-4.6 to 3.3)	2.1 (-1.6 to 5.8)	-1.1 (-3.9 to 1.9)	-0.2 (-3.0 to 2.7)	0.9 (-0.6 to 2.3)	0.9 (-0.6 to 2.3)	-0.2 (-1.2 to 0.9)	-0.1 (-1.1 to 1.0)

*Results represent percent difference in biomarker values per interquartile range change in PM estimates. The 1, 3 and 12 month IQRs were 7.26, 6.23, 5.26 $\mu g/m^3$ for $PM_{2.5}$, 11.25, 9.27, 7.85 $\mu g/m^3$ for PM_{10}, and 6.10, 5.89, 5.51 $\mu g/m^3$ for $PM_{2.5-10}$, respectively.

[a]Basic models adjusted for age, season, smoking status, menopausal status and postmenopausal hormone use

[b]Full models additionally adjusted by BMI, physical activity, alcohol consumption, MI family history, high blood pressure, Alternate Healthy Eating Index, pack yrs smoked, and census-tract median household income

Table 5. Percent differences and 95% confidence intervals of markers of inflammation by distance to road category

	CRP	IL-6	Fibrinogen	ICAM-1
Basic[a]				
≥200m	---	---	---	---
50-199m	-0.5 (-9.1 to 9.0)	7.7 (0.7 to 15.1)	1.8 (-1.6 to 5.4)	-0.4 (-2.8 to 2.0)
0-49m	14.1 (2.5 to 27.0)	8.4 (0.1 to 17.4)	1.4 (-2.5 to 5.5)	-1.9 (-4.7 to 1.0)
Full[b]				
≥200m	---	---	---	---
50-199m	0.7 (-7.4 to 9.3)	8.4 (1.7 to 15.6)	1.5 (-1.9 to 4.9)	-0.4 (-2.8 to 2.1)
0-49m	13.1 (2.6 to 24.6)	6.7 (-1.1 to 15.2)	0.8 (-3.0 to 4.8)	-1.9 (-4.7 to 1.0)

*Results from distance to road models represent the percent difference in biomarker values in comparison to study participants ≥200m from a roadway. Nearest road was defined as the distance from the residence to the closest A1-A3 category road as defined by U.S. Census Bureau, 2014. A1 (primary roads, typically interstate highways, with limited access, division between the opposing directions of traffic, and defined exits); A2 (primary major, non-interstate highways and major roads without access restrictions); A3 (smaller, secondary roads, usually with more than two lanes)

[a]Basic models adjusted for age, season, smoking status, menopausal status and postmenopausal hormone use

[b]Full models additionally adjusted by BMI, physical activity, alcohol consumption, MI family history, high blood pressure, Alternate Healthy Eating Index, pack yrs smoked, and census-tract median household income

Supplemental Tables

Table S1. Descriptive characteristics of 2946 NHS participants with one or more assayed biomarker measurement by individual nested case-control study

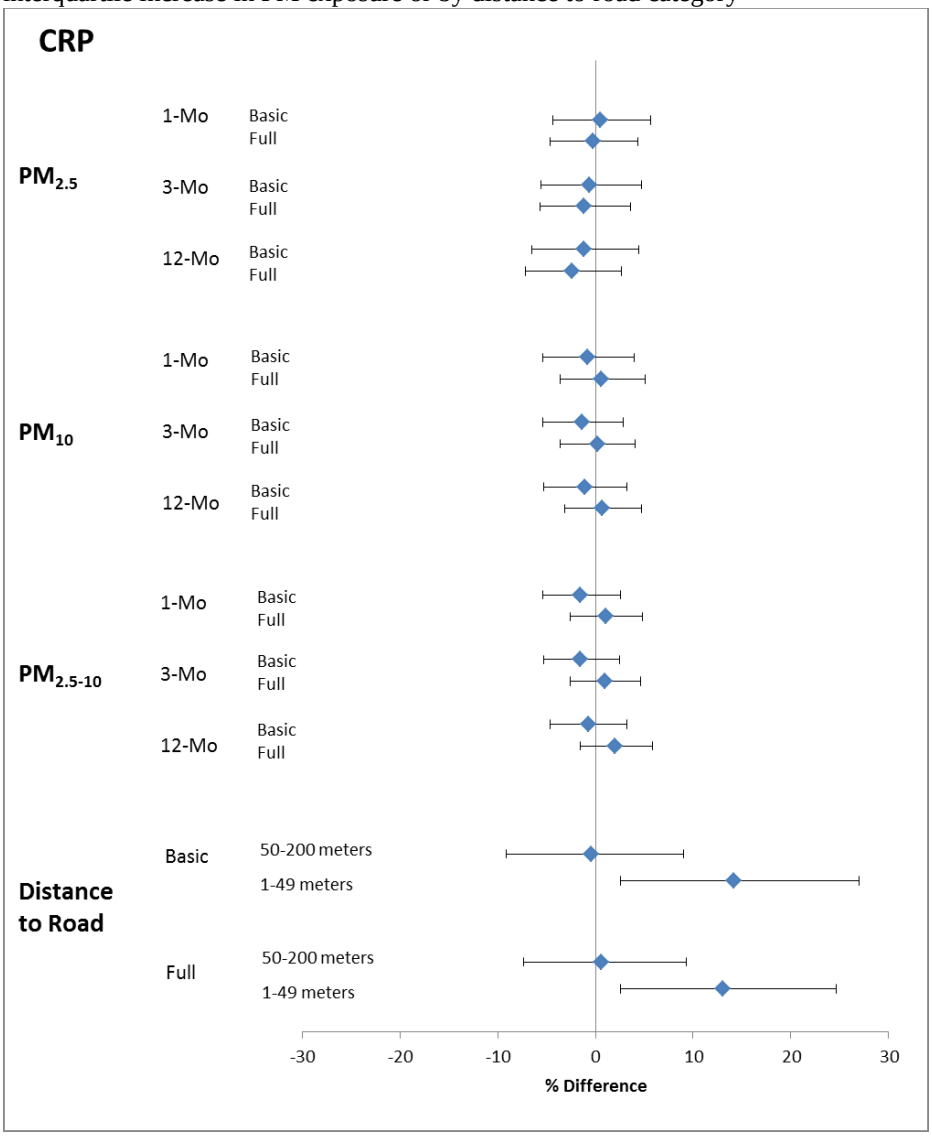
Characteristics	MI Study (N=486)	MD Study (N=462)	BC Study (N=1238)	DB Study (N=760)
BMI [kg/m2 (mean ± SD)]	25.3 (4.8)	25.1 (4.7)	25.64 (4.8)	26.6 (6.3)
Age [Yrs]	60.7 (6.5)	63.1 (4.7)	58.6 (8.3)	56.3 (7.8)
median household income (mean ± SD)	65,441 (26,692)	65,003 (25,783)	65,415 (25,721)	65,776 (27,643)
Pack-years of smoking (mean ± SD)[a]	29.4 (22.6)	23.3 (20.9)	23.2 (19.5)	22.4 (18.9)
Smoking status (%)				
Current	31.2	6.0	11.8	11.9
Past	33.9	43.0	40.6	41.0
Never	34.9	50.4	47.3	46.3
Missing	0.0	0.6	0.3	0.8
Region (%)				
Northeast	48.6	43.6	48.4	45.8
Midwest	20.5	18.4	20.3	22.1
West	15.0	17.5	15.8	14.5
South	15.9	20.5	15.4	17.7
Alcohol category (%)				
Non-drinker (0 g/day)	17.9	20.7	19.7	16.9
0.1+ g/d	78.1	76.7	78.2	80.1
Missing	4.1	2.6	2.1	3.0
Physical activity (%)				
<3 MET hr/week	21.5	19.4	20.2	21.4
3 to < 18 MET hr/week	49.1	41.9	46.2	48.4
18+ MET hr/week	28.6	38.5	32.9	28.5
Missing	0.8	0.2	0.6	1.7
Postmenopausal hormone use %				
Never or Past Use	50.3	55.3	49.0	42.1
Current use	36.7	38.0	33.7	35.2
Premenopausal	11.8	4.5	16.8	22.4
Unknown	1.2	2.1	0.5	0.4
MI Family History %	36.1	40.8	37.7	34.1
High Blood Pressure %	29.2	36.3	28.8	29.3
Season of Blood Draw[b]				
Winter	20.7	20.6	23.6	23.8
Spring	16.8	24.1	20.8	24.4
Summer	37.4	30.4	28.8	27.5
Fall	25.1	25.0	26.8	24.3

[a] Among former and current smokers only

[b] blood draw in Dec-Feb defined as winter, Mar-May as spring, Jun-Aug as summer, Sep-Nov as Fall)

Figures

Figure 1. Percent differences and 95% confidence intervals in C-reactive protein (CRP) per interquartile increase in PM exposure or by distance to road category



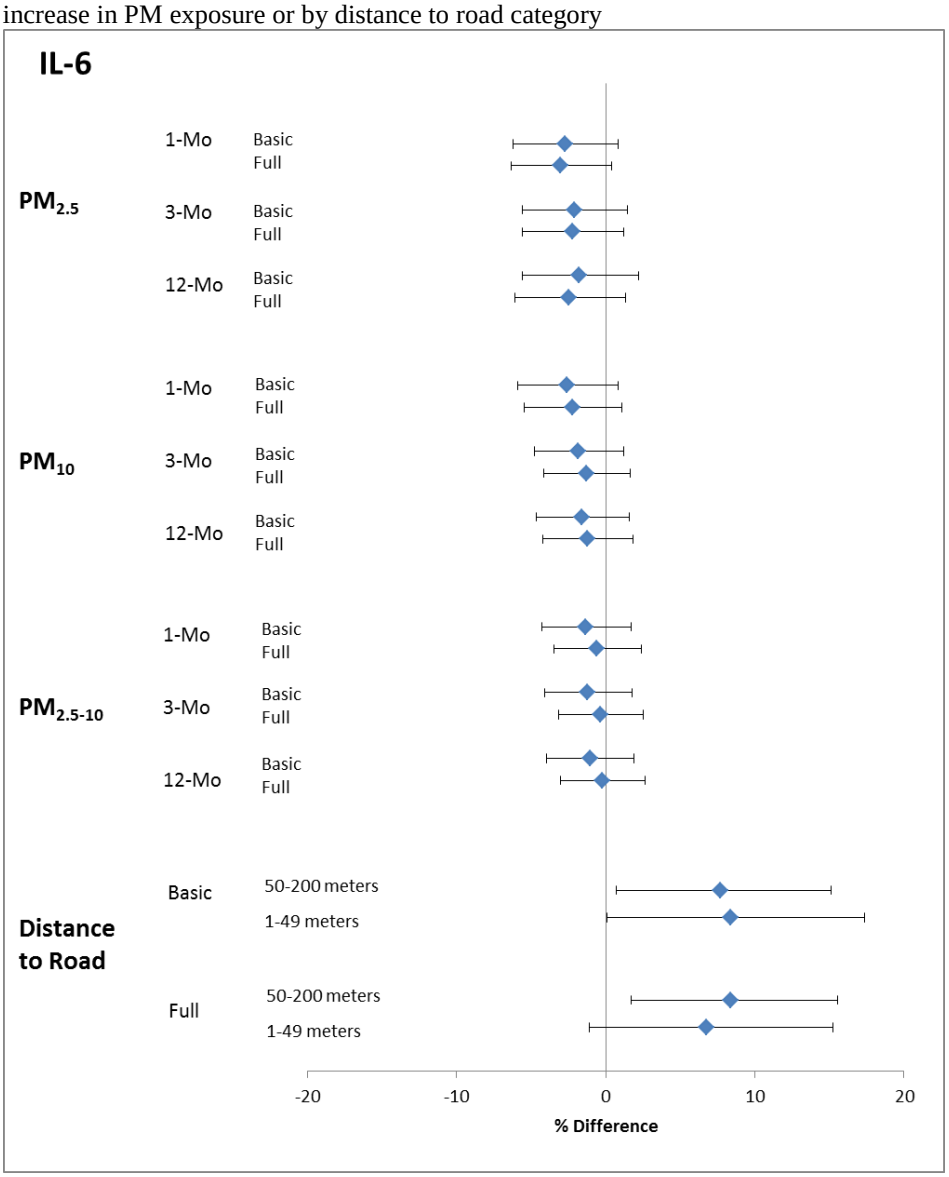
*PM model results represent percent difference in biomarker values per interquartile range change in PM estimates. The 1, 3 and 12 month IQRs were 7.26, 6.23, 5.26 µg/m^3 for $PM_{2.5}$, 11.25, 9.27, 7.85 µg/m^3 for PM_{10}, and 6.10, 5.89, 5.51 µg/m^3 for $PM_{2.5-10}$, respectively.

* Results from distance to road models represent the percent difference in biomarker values in comparison to study participants ≥200m from a roadway. Nearest road was defined as the distance from the residence to the closest A1-A3 category road as defined by U.S. Census Bureau, 2014. A1 (primary roads, typically interstate highways, with limited access, division between the opposing directions of traffic, and defined exits); A2 (primary major, non-interstate highways and major roads without access restrictions); A3 (smaller, secondary roads, usually with more than two lanes)

[*]Basic models adjusted for age, season, smoking status, menopausal status and postmenopausal hormone use

[*]Full models additionally adjusted by BMI, physical activity, alcohol consumption, MI family history, high blood pressure, Alternate Healthy Eating Index, pack yrs smoked, and census-tract median household income

Figure 2. Percent differences and 95% confidence intervals in interleukin-6 (IL-6) per interquartile increase in PM exposure or by distance to road category



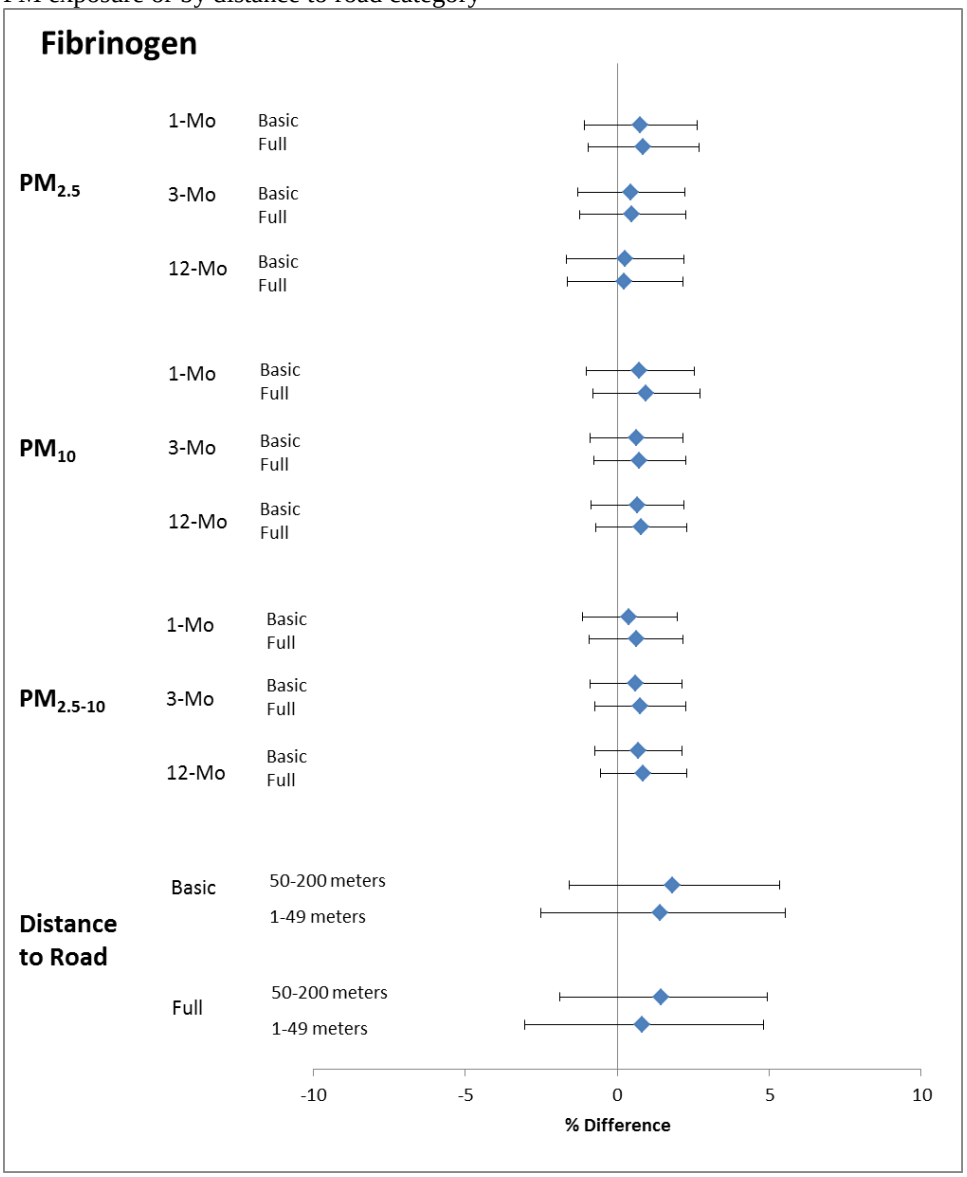
*PM model results represent percent difference in biomarker values per interquartile range change in PM estimates. The 1, 3 and 12 month IQRs were 7.26, 6.23, 5.26 µg/m^3 for $PM_{2.5}$, 11.25, 9.27, 7.85 µg/m^3 for PM_{10}, and 6.10, 5.89, 5.51 µg/m^3 for $PM_{2.5-10}$, respectively.

* Results from distance to road models represent the percent difference in biomarker values in comparison to study participants ≥200m from a roadway. Nearest road was defined as the distance from the residence to the closest A1-A3 category road as defined by U.S. Census Bureau, 2014. A1 (primary roads, typically interstate highways, with limited access, division between the opposing directions of traffic, and defined exits); A2 (primary major, non-interstate highways and major roads without access restrictions); A3 (smaller, secondary roads, usually with more than two lanes)

[*]Basic models adjusted for age, season, smoking status, menopausal status and postmenopausal hormone use

[*]Full models additionally adjusted by BMI, physical activity, alcohol consumption, MI family history, high blood pressure, Alternate Healthy Eating Index, pack yrs smoked, and census-tract median household income

Figure 3. Percent differences and 95% confidence intervals in fibrinogen per interquartile increase in PM exposure or by distance to road category



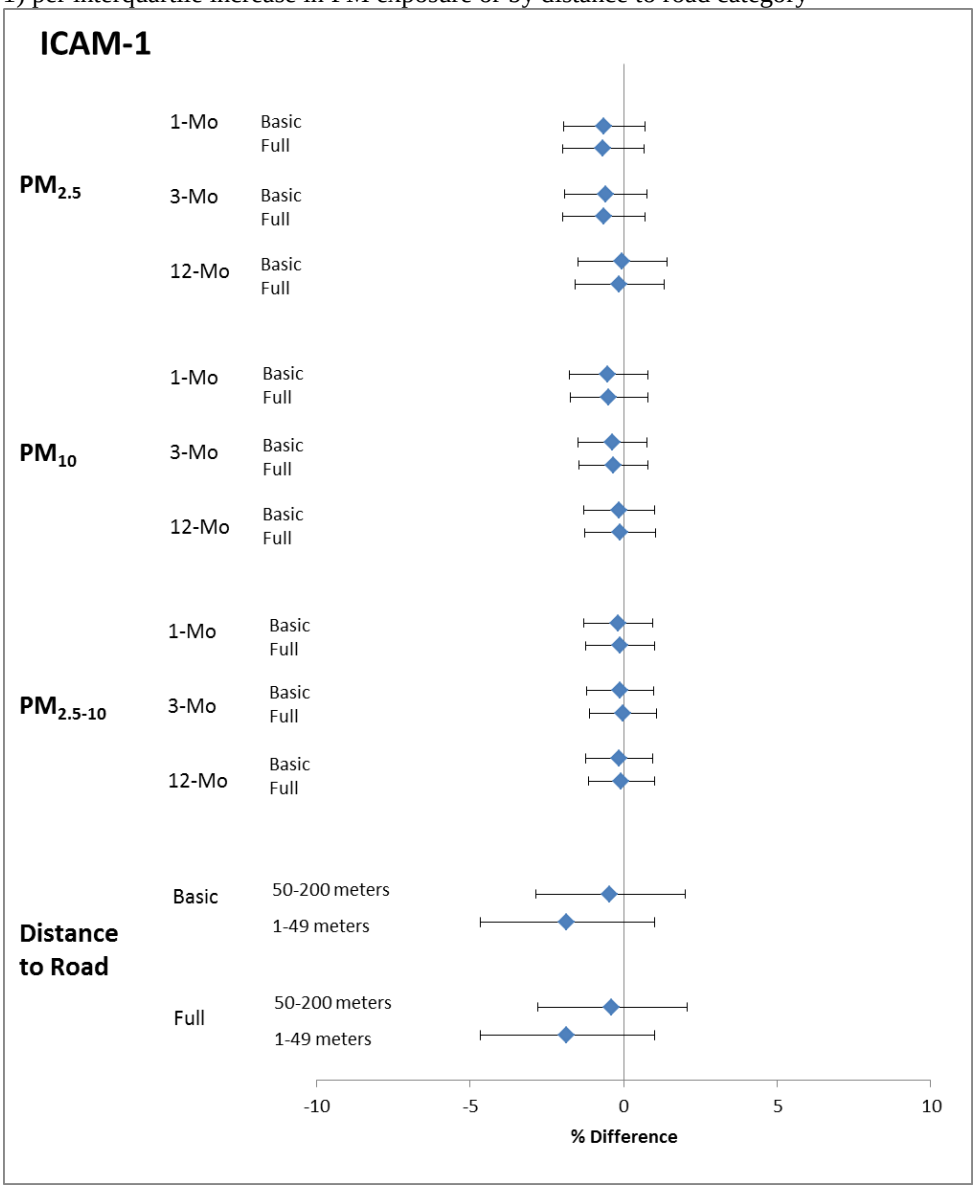
*PM model results represent percent difference in biomarker values per interquartile range change in PM estimates. The 1, 3 and 12 month IQRs were 7.26, 6.23, 5.26 µg/m^3 for $PM_{2.5}$, 11.25, 9.27, 7.85 µg/m^3 for PM_{10}, and 6.10, 5.89, 5.51 µg/m^3 for $PM_{2.5-10}$, respectively.

* Results from distance to road models represent the percent difference in biomarker values in comparison to study participants ≥200m from a roadway. Nearest road was defined as the distance from the residence to the closest A1-A3 category road as defined by U.S. Census Bureau, 2014. A1 (primary roads, typically interstate highways, with limited access, division between the opposing directions of traffic, and defined exits); A2 (primary major, non-interstate highways and major roads without access restrictions); A3 (smaller, secondary roads, usually with more than two lanes)

[*]Basic models adjusted for age, season, smoking status, menopausal status and postmenopausal hormone use

[*]Full models additionally adjusted by BMI, physical activity, alcohol consumption, MI family history, high blood pressure, Alternate Healthy Eating Index, pack yrs smoked, and census-tract median household income

Figure 4. Percent differences and 95% confidence intervals in intra-cellular adhesion molecule (ICAM-1) per interquartile increase in PM exposure or by distance to road category



*Results from PM models represent percent difference in biomarker values per interquartile range change in PM estimates. The 1, 3 and 12 month IQRs were 7.26, 6.23, 5.26 μg/m^3 for $PM_{2.5}$, 11.25, 9.27, 7.85 μg/m^3 for PM_{10}, and 6.10, 5.89, 5.51 μg/m^3 for $PM_{2.5-10}$, respectively.

*Results from distance to road models represent the percent difference in biomarker values in comparison to study participants ≥200m from a roadway. Nearest road was defined as the distance from the residence to the closest A1-A3 category road as defined by U.S. Census Bureau, 2014. A1 (primary roads, typically interstate highways, with limited access, division between the opposing directions of traffic, and defined exits); A2 (primary major, non-interstate highways and major roads without access restrictions); A3 (smaller, secondary roads, usually with more than two lanes)

*Basic models adjusted for age, season, smoking status, menopausal status and postmenopausal hormone use

*Full models additionally adjusted by BMI, physical activity, alcohol consumption, MI family history, high blood pressure, Alternate Healthy Eating Index, pack yrs smoked, and census-tract median household income

Chapter 6: Conclusions

The results from our two studies of short-term PM exposures and stroke advance the current literature in several ways. Our findings in the HPFS are consistent with a recent review and meta-analysis that show PM exposures are associated with ischemic stroke but not hemorrhagic stroke (Wang et al. 2014) Contrasting the findings between the ischemic and hemorrhagic stroke results also provide more evidence that these two major stroke subtypes should be treated as heterogeneous outcomes in analyses related to air pollution, a point stressed by Ljungman and Mittleman, 2014 (Ljungman and Mittleman 2014). Our HPFS study also may be the first to suggest that those without a history of high cholesterol or those not currently taking aspirin are at elevated risk for acute PM-related ischemic stroke events. Though we found no evidence of an association between $PM_{2.5}$ exposure and total cerebrovascular, ischemic, or hemorrhagic stroke hospitalizations in Maryland, this study is one of the first to examine potential effect modifiers of hemorrhagic stroke.

In both case-crossover studies, the examination of risk by potential effect modifiers was of major interest, as identifying those most vulnerable to the effects of ambient particulate exposures may inform future public health prevention policies. However, our findings of elevated associations among non-aspirin users are novel and should first be repeated among other study populations. As regular aspirin use was elevated among those with a previous history of high blood pressure and hypertension, additional studies may help shed light on whether our results are indicative of higher

risk from PM-related stroke among those without history of hypertension or hypercholesterolemia, or whether medication use may potentially lower or eliminate PM-related ischemic stroke risk. It should also be noted that care is advised in the prophylactic use of aspirin in regards to stroke (Lei et al. 2016). Though aspirin may lower risk of ischemic stroke, some evidence suggests it may increase the risk of hemorrhagic stroke (Lei et al. 2016).

As the US EPA re-evaluates regulations on the criteria pollutants every five years under The Clean Air Act, results from these studies may directly contribute to future policy actions. The NAAQS were last revised in 2012 with a major change being that annual $PM_{2.5}$ standards were divided into a primary (12 $\mu g/m^3$) and secondary (15$\mu g/m^3$) standard (US EPA 2017c). Primary standards are meant to provide public health protection to sensitive populations (asthmatics, children, elderly), while secondary standards provide public welfare protection (decreased visibility, damage to animals crops). Currently there is no division between primary and secondary standards for 24-hour standards for $PM_{2.5}$ (35 $\mu g/m^3$) or PM_{10} (150 $\mu g/m^3$). The short-term mean exposures for $PM_{2.5}$ and PM_{10} in both of our PM-stroke studies did not exceed the NAAQS standards. Our significant findings between PM_{10} exposures and ischemic stroke occur at PM levels within the current standards set by EPA. Thus, our findings may influence future risk and policy assessment documents. We expect this work to be incorporated into the next PM Integrative Science Assessment (ISA) document that provides a comprehensive review, synthesis, and evaluation of the most policy-relevant science on the health effects of PM exposure. Following the

EPA NAAQS review process, the ISA is then used as the scientific backbone for developing the Risk/Exposure Assessment (REA) which quantifies the associated risk to human health (US EPA 2017d). The ISA, REA, and a Policy Assessment are then used in final rulemaking.

Our third study in the NHS was intended to inform hypothesized pathophysiological pathways of how PM exposure may alter future cerebrovascular and cardiovascular risk. The fractions of particulate air pollution exposures we examined were not associated with the markers of inflammation. However, after adjusting for potential confounding variables, concentrations of both CRP and IL-6 were elevated among participants living close to a major roadway compared to those living further away. This finding provides some evidence that proximity to traffic may influence inflammatory markers of cardiovascular risk. We also found higher effect estimates among coarse PM models compared to fine PM models. These findings deserve further investigation. Additional studies are needed to determine if the positive association with proximity to major roadway seen in our study is influenced by PM exposures of certain size fractions, specific constituents, or other gaseous pollutants associated with traffic. Such studies would also benefit from the inclusion of daily exposures. Including both short and long-term exposures in the same models may provide evidence on how exposures of varying durations influence inflammatory pathways. Studies examining mechanistic pathways are a crucial piece to establishing causal pathways between exposure and subsequent health outcomes. Work, such as this, provide a crucial ‘how’ to the studies of association between exposure and

disease. The EPA incorporates such work in their integrated science assessment (ISA) documents that provide a comprehensive review, synthesis and evaluation of the most policy-relevant science. These documents directly inform future policy and air quality regulations.

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