AIR POLLUTION AND CARDIOVASCULAR DISEASE: FROM THE PERSPECTIVES OF BOTH SHORT- AND LONG-TERM EXPOSURE

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ABSTRACT

One of the potential risk factors for CVD is ambient air pollution. This dissertation consists of three manuscripts, in which we examined the association between both short- and long-term air pollution exposure and clinical and subclinical CVD.

The first manuscript investigated the short-term effect of multiple pollutants, $PM_{2.5}$, O_3, NO_2 and SO_2 on CVD emergency room visits in Allegheny County, PA in 1999 – 2011, using a case-crossover design. We found that per IQR increase of O_3 exposure (25.52 ppb), there was 6.6% (95% CI: 0.8% - 12.7%) increase in the odds of an acute myocardial infarction (AMI) emergency room visit. Among women and Blacks, we observed the association between $PM_{2.5}$ and AMI. This association persisted in analyses stratified by age, race, gender, season, as well in the later years with lower exposure levels. There was also a suggestive association between $PM_{2.5}$ and NO_2 and peripheral vascular disease.

The second manuscript examined the long-term exposure to $PM_{2.5}$ and O_3 over five years as a predictor for subclinical atherosclerosis measured approximately seven years later in a cohort among mid-life women from the multicenter multiethnicity cohort study, the Study of Women's Health Across the Nation (SWAN). Among these 1188 women, a 1 $\mu g/m^3$ higher yearly cumulative exposure to $PM_{2.5}$ over 5 years was associated with an 8.0 μm (95% CI: 1.0 – 15.1) greater mean of maximum CCA IMT at later mid-life.

The third manuscript was a prospective study that examined the association between $PM_{2.5}$ and O_3 and progression of subclinical atherosclerosis during a 2-year follow-up at only two sites, Pittsburgh and Chicago of SWAN. In the primary analysis, a 1 $\mu g/m^3$ higher yearly mean exposure to $PM_{2.5}$ during the follow-up was associated with a 4.28 (95% CI: 0.02 – 8.54) μm per year increase of mean of maximum CIMT progression, after adjusting for confounders. Yearly mean exposure to $PM_{2.5}$ during the follow-up contributed to plaque index progression adjusting for socioeconomic factors, but not in the fully adjusted model.

These findings provide additional evidence that both short- and long-term exposure to air pollution may have significant deleterious effects on heart health. This is of public health significance because the reduction of the ambient air pollution is at the policy level. These evidences imply further regulations needed for public health benefit.

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PREFACE

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1.0 INTRODUCTION

For the past twenty years, researchers have become aware that ambient air pollution may be responsible for a large number of cardiovascular and respiratory events (Atkinson et al., 2013; R. D. Brook et al., 2010; Franchini & Mannucci, 2012). These effects have been seen globally. According to the World Health Organization (WHO), there are about 3.7 million deaths attributed to ambient air pollution each year globally, and the majority of which are stroke, ischemic heart disease, and respiratory disease (World Health Organization, 2014b).

Air pollution is ubiquitous, and exists in our everyday environment. It is not easy to avoid and at high levels has a deleterious effect on health (Lim, Vos et al. 2012). One of the most important organ systems that can be affected by air pollution is the cardiovascular system (Atkinson, Carey et al. 2013, World Health Organization 2014, World Health Organization 2014). Air pollution has been shown to be related to oxidative stress, systemic inflammation, and endothelial cell dysfunction (Sun, Wang et al. 2005, Chuang, Chan et al. 2007, Bind, Baccarelli et al. 2012, Rich, Kipen et al. 2012, Hajat, Allison et al. 2015), which are all in the etiological pathway of atherosclerosis. The clinical consequence of atherosclerosis is cardiovascular disease (CVD), which is the most common disease among adults in the U.S. There are about 83.6 million American adults with some form of CVD, including coronary heart disease and stroke (Go, Mozaffarian et al. 2014). Heart disease is the number one cause of death in the U.S., while stroke

is the fifth (Centers for Disease Control and Prevention 2015). However, there is currently not enough evidence linking air pollution and atherosclerosis in epidemiology studies.

Studies to date on air pollution have considered mortality and morbidity related to hospitalizations (Dominici, Peng et al. 2006, Bell, Ebisu et al. 2008, Peng, Bell et al. 2009, Boehm Vock, Reich et al. 2015). There are very few studies that have examined the long-term association between air pollution and CVD (Miller, Siscovick et al. 2007, Hoek, Krishnan et al. 2013); furthermore, there are few studies in humans which have considered subclinical atherosclerosis markers and air pollution either in cross-sectional or longitudinal studies (Künzli, Jerrett et al. 2010, Adar, Sheppard et al. 2013, Su, Hwang et al. 2015). The studies addressing the short-term exposure of air pollution and CVD, usually used mortality and hospitalization data (Ballester, Rodriguez et al. 2006, Gill, Curl et al. 2011, Kloog, Nordio et al. 2014, Milojevic, Wilkinson et al. 2014, Talbott, Rager et al. 2014, Chang, Chen et al. 2015). Using mortality data may not necessarily capture the exact date when patients needed medical aid, as people may be hospitalized for days before the event. While using the hospitalization data, it is difficult to distinguish the acute condition rather than a scheduled hospitalization or surgery. Thus, my objectives in this section are 1) to present a literature review using emergency room visits for a large area to examine the acute effect of $PM_{2.5}$, ozone (O_3) and other criteria gaseous pollutant and possible $PM_{2.5}$ constituents on cardiovascular admissions using time series analysis; 2) to present an overview of the literature describing the relationship between air pollution and sub-clinical atherosclerosis specifically considering the effects of $PM_{2.5}$ and O_3; and 3) to propose the studies I will carry out in these two areas to fill in the gaps in the current literature.

1.1 AMBIENT AIR POLLUTION

Ambient air pollution is not a single exposure agent, but a mixture. The National Ambient Air Quality Standards (NAAQS) (U.S. Environmental Protection Agency 2014) established by the Environmental Protection Agency (EPA) set criteria for six major air pollutants: carbon monoxide (CO), lead (Pb), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), O_3, and particulate matter (PM) . But, there are many more pollutants in the atmosphere, and most of them have adverse effects on human health. For instance, volatile organic compounds (VOCs) are a family of thousands of chemicals, which include carcinogens such as benzene, formaldehyde, and ethylbenzene and persistent organic compounds (POPs). These are toxic and resist degradation in the natural environment.

The two major air quality indicators identified by EPA are $PM_{2.5}$ and O_3 (AirNow 2016). The current literature review will focus on these two pollutants as well as their long-term and short-term effects on subclinical atherosclerosis and CVD emergency room visits.

1.1.1 $PM_{2.5}$

$PM_{2.5}$ (aerodynamic diameter less than 2.5 μm) is both a primary and secondary air pollutant (World Health Organization 2013). Primary air pollutant refers to compounds that are emitted to the air directly. While secondary air pollutants are those in which the compound is formed in the atmosphere from other air pollutants. The major sources of $PM_{2.5}$ are combustion processes (e.g. road vehicles, power plants, steel mills, etc.) and certain industry processes (e.g. mining, construction, manufacturing bricks and ceramic, etc.). Also, $PM_{2.5}$ can be formed by other gaseous pollutants. SO_2 and NO_2 from road vehicles, or industry emission and ammonia from

fertilizers can easily form $PM_{2.5}$, and this is the major component of the inorganic part of $PM_{2.5}$. Through these complicated combustion and industrial processes and formation from other chemicals, it is easy to understand that the $PM_{2.5}$ is a mixture of all chemicals that possibly exist in the air. Furthermore, given the small size of $PM_{2.5}$, the relative surface area of these particles is big. Thus, there are additional chemicals that can attach to the particles. In summary, $PM_{2.5}$ is not one pollutant alone, but a very complex mixture that can harm human health in several ways through several mechanisms.

Particles in the range of 0.1 – 2 μm, also have the longest lifetime in the atmosphere compared to particles with larger diameters (Rao, Frank et al. 2003). The lifetime of $PM_{2.5}$ is about one week, and the major form of deposition procedure is by rain. The particles smaller than this range are more likely to be condensed and coagulated to form $PM_{2.5.}$ Particles larger than 2.5 μm have a higher dry deposition rate, and thus, the lifetime is much shorter (about 1 day). Therefore, $PM_{2.5}$ is persistent in the environment compared to particles of other sizes.

With the small size, $PM_{2.5}$ can penetrate deeply into the lungs and reach bronchioles and alveoli (U.S. Environmental Protection Agency 2014). PM can, then, play a harmful role in human health. The most significant health impacts of PM are to the respiratory and cardiovascular systems after both long-term and short-term exposure (Scovronick, Adair-Rohani et al. 2015). There are three possible pathophysiological pathways proposed by Brook et al: 1) PM can trigger oxidative stress and inflammation, causing endothelial cell dysfunction, thrombosis, and coagulation; 2) it can penetrate to blood vessels and affect the endothelial cells, platelet aggregation, and blood pressure; 3) it also can trigger autonomic nervous system (ANS) imbalance, and then cause endothelial dysfunction (Brook, Rajagopalan et al. 2010). The first two pathways can lead to atherosclerosis (Künzli, Perez et al. 2011, World Health Organization

2013), and at a very high dose, it will lead to emergency room visits or mortality. Additionally, ANS imbalance can modulate cardiac arrhythmias, which can result in short-term CVD events (Shen and Zipes 2014). Besides the mechanisms proposed above, there is another potential pathway proposed that links PM to CVD. In several animal studies, researchers used APOE or low-density lipoprotein receptor (LDLR) knock-out mice to show a 50% increase in plaque volume after exposure to $PM_{2.5}$ (Sun, Wang et al. 2005, Araujo, Barajas et al. 2008, Soares, Carvalho-Oliveira et al. 2009). Both the APOE and LDLR knock-out mice are well established animal models of hypercholesterolemia after a high fat diet and subsequent atherosclerosis (Nakashima, Plump et al. 1994, Radonjic, Wielinga et al. 2013). This may indicate that long-term $PM_{2.5}$ may induce inflammatory response in adipose tissue, and therefore, lead to atherosclerosis.

1.1.2 Ozone (O_3)

Ozone (O_3), a secondary pollutant, is a high-powered form of oxygen, which consists of three atoms of oxygen. O_3 is formed by NOx (the NOx family consists of NO_2 and NO, which have a fast chemical reaction cycle to form each other) and VOCs by photolysis (U.S. Environmental Protection Agency 2015). NOx and VOCs are emitted by factories, power plants, and road traffic (AirNow 2015). Besides this, the strength of the ultra-violet is can influence how much O_3 is formed. Exposure to O_3 can cause cough, shortness of breath and irritation to throat and lung(U.S. Environmental Protection Agency 2016). O_3 can also exacerbate the condition of asthma patients. Furthermore, a few studies have indicated (Franchini and Mannucci 2012, Carlsen, Forsberg et al. 2013, Goodman, Prueitt et al. 2015) that O_3 might also increase the level of biomarkers related to CVD (e.g. circulating level of inflammatory markers, such as CRP and

IL-6, marker of thrombosis PAI-1 and the oxidative stress marker 8-hydroxy-2'-deoxyguanosine (8-OHdG)), and CVD hospital admission and death (Goodman, Prueitt et al. 2014, Goodman, Prueitt et al. 2015). O_3 is a very strong oxidizing agent, that can produce free radicals in the system, thus increasing oxidative stress and damage to different systems in the body (Yang and Omaye 2009, Lobo, Patil et al. 2010, U.S. Environmental Protection Agency 2016). For instance, it can oxidize LDL-c, which is atherogenic.

According to EPA, the level of O_3 exposure has been decreasing over time (U.S. Environmental Protection Agency 2016). However, this trend may be due to measurement bias, because in the earlier years there was limited data for year-round O_3 monitoring, especially in the north, where there is a lower ultraviolet index. In recent years, most places in the U.S. have yearlong O_3 monitoring data, which has added more information from the north, resulting in this decreasing trend. Therefore, in some regions or during certain seasons, the O_3 level has not necessarily decreased over this time period (Simon, Reff et al. 2014).

1.1.3 Air Quality Monitoring and Standard

U.S. EPA monitored and regulated seven air pollutant in the atmosphere, PM_{10}, $PM_{2.5}$, O_3, NO_2, SO_2, CO and lead (Pb). The standard monitoring measures for these pollutants were collected by two systems, the State and Local Air Monitoring Stations and the National Air Monitoring Station Network, and reported to EPA. These monitors were targeted at areas with high pollution concentrations and population density (Hall, Eyth et al. 2012). National Ambient Air Quality Standards (NAQQS) set standard for these pollutants for both the acute effect (standard for 1-hour, 3-hour, 8-hour, or 1 day) and the chronic effect (standard for 1-year average). Pollution level based on these standards were calculated and available in the Air Quality System DataMart,

which is managed by EPA (U.S. Environmental Protection Agency 2017). The source of air pollution data for this dissertation was mainly derived from these monitors from this source.

Besides the monitor data, many studies also applied the modeled data to estimate the air pollution exposure in the study population (Adar, Sheppard et al. 2013, Perez, Wolf et al. 2015, Su, Hwang et al. 2015). Using modeled data can eliminate some limitations from the monitor data, like the missingness of the monitor data when the monitor was malfunctioning or the monitor only operated for a certain period of the time during the year (Hall, Eyth et al. 2012). Moreover, the spatial variation on a finer scale may present in the study area (Tunno, Michanowicz et al. 2015), which cannot be captured by the regulatory monitors.

One of the most commonly used methods for air pollution exposure modeling is land-use regression (LUR), which implementing tens or hundreds of monitors at the study site to capture spatial variation (Hoek, Beelen et al. 2008, Adar, Sheppard et al. 2013, Perez, Wolf et al. 2015). The usual protocol was to install the monitors for a two-week period, and at least for two seasons (warm and cold) (Kaufman, Adar et al. 2012), and then, use the meteorological (e.g. temperature, pressure, humidity, etc.) and land cover (e.g. elevation, buildings, residential or business coverage, etc.) variables as the predictors to establish the model. The spatial variation was determined by this model, and the temporal extrapolation to the whole study period is based on regulatory monitors (Hoek, Beelen et al. 2008). Another widely used method for the spatio-temporal modeling for $PM_{2.5}$ was aerosol optical depth (AOD), which applied satellite data to estimate the ground-level particle exposure level (Kloog, Koutrakis et al. 2011). The satellite data for AOD is available from the National Aeronautics and Space Administration (NASA) for every day. This method can capture the spatial and temporal variance simultaneously. Besides

these two methods, there were other models that have been applied in the epidemiological studies (Künzli, Jerrett et al. 2010, Sun, Kaufman et al. 2013, Kim, Sheppard et al. 2014).

1.2 BENEFIT OF AIR QUALITY REGULATION

The Clean Air Act was signed by President Nixon in 1977 to address the deleterious effects of air pollution (U.S. Environmental Protection Agency 2017). A series of air quality regulations have been implemented in the U.S. by EPA during the past few decades, and as a consequence, the air quality has improved (Dominici, Peng et al. 2007). According to a 2011 report on the costs and benefits of regulations, the regulations implemented by EPA lead to the biggest benefit to the U.S. population (Office of Management and Budget 2011). These regulations, resulting in 20 rules designated by the Office of Air, had an estimated cost of about 21 billion dollars, and yielded a benefit of up to 500 billion dollars. A report from Aphekom project in Europe indicated that life expectancy would increase for people 30 years old and older by following compliance with the WHO's air quality standard for $PM_{2.5}$ of 10 $\mu g/m^3$. Even reducing the annual $PM_{2.5}$ level from 12 $\mu g/m^3$ to 10 $\mu g/m^3$, would lead to a 2.2-month increase in life expectancy. In the U.S., most places had an average $PM_{2.5}$ level around 14 $\mu g/m^3$ in 2000, and dropped about 37% over the following 15 years, decreasing to around 10 $\mu g/m^3$ in 2015. However, this level may not be sufficiently low in terms of cardiovascular health. A study in the Northeast of the U.S. and restricted the areas to yearly level less than 10 $\mu g/m^3$, and they still revealed an association between air pollution and clinical CVD events (Shi, Zanobetti et al. 2016). The potential threshold effect from this study was about 6 $\mu g/m^3$, which is the "background" level for rural areas.

1.3 CARDIOVASCULAR DISEASE

1.3.1 Cardiovascular Clinical Outcomes

CVD is the leading cause of death in the U.S. and worldwide (Centers for Disease Control and Prevention 2015, World Health Organization 2015). There are about 28.4 million (11.7%) adults diagnosed with all types of heart disease (coronary heart disease, angina, heart attack, or any other heart condition or disease) in the U.S. (Centers for Disease Control and Prevention 2017). The age-adjusted prevalence of heart disease is slightly higher among the male than the female (12.2% vs. 10.0%). The age-adjusted prevalence of heart disease is also higher among White (11.3%) and American Indian or Alaska Native (13.7%), and relatively lower among Black or African American (13.7%) and other racial groups. However, Black or African American have highest prevalence of hypertension (34.4%), while all the other racial groups have a prevalence lower than 30%. And, the prevalence is also higher among the population with lower education, unemployed but has worked previously and poor or near poor. Each year, the American Heart Association publishes the updated statistics for heart disease and stroke morbidity and mortality, as well as risk factors contributing to CVD (Mozaffarian, Benjamin et al. 2016). According to these statistics, improvement in the monitoring and management of CVD risk during the past decades has led to a decrease in CVD mortality and morbidity (Lloyd-Jones, Adams et al. 2010, Mozaffarian, Benjamin et al. 2016). However, the burden of CVD, including associated economic cost, remains high with an $193.1 billion healthcare related expenses and $123.5 billion due to premature mature deaths of CVD in 2011 – 2012.

Since the Framingham Heart Study, numerous CVD risk factors have been identified (Kannel and McGee 1979, Hubert, Feinleib et al. 1983, Benjamin, Levy et al. 1994). Traditional

CVD risk factors have been well studied and documented by population based cohort studies, including social factors (like socioeconomic status, education, poverty and inequality), behavioral factors, (like poor diet, physical inactivity, smoking, alcohol, and therefore, obesity), biological factors (like elevated blood lipids, high blood pressure, elevated blood glucose/diabetes, thrombosis and inflammation) and family history (Tzoulaki, Elliott et al. 2016). Most of these factors have been considered as modifiable factors, and preventive measures have been implemented to help reduce the CVD burden (Mozaffarian, Wilson et al. 2008, Batacan, Duncan et al. 2015). However, there is still a significant proportion of CVD risk unexplained by these established risk factors; ambient air pollution is one of the more novel risk factors related to CVD (Franklin, Brook et al. 2015).

1.3.2 Atherosclerosis

Atherosclerosis is an inflammatory and lipids driven process, in which plaque builds up in the artery (Lu and Daugherty 2015). In order to study CVD before an event occurs, biomarkers of subclinical atherosclerosis as outcomes have been widely adopted in research. Epidemiologic studies have increasingly used biomarkers of subclinical atherosclerosis as an outcome, especially carotid intima-media thickness (CIMT) and plaque via B-mode ultrasound and CAC via computed tomography (CT). Atherosclerosis is a life-long progression, the progression of atherosclerosis may better capture the atherogenic risk of the study population (Künzli, Perez et al. 2011). Early vascular change can be reflected by progression of CIMT (Lorenz, Polak et al. 2012).

CIMT, a surrogate biomarker of atherosclerosis, can predict CVD and stroke events (Chambless, Folsom et al. 2000, Bots, Evans et al. 2003, Bauer, Caviezel et al. 2012, van den

Oord, Sijbrands et al. 2013). Evidence from additional investigations also demonstrated that CIMT is a validated biomarker for atherosclerosis in young populations, who did not develop any signs of significant atherosclerosis, like plaque or coronary arterial calcification (CAC) (Geerts, Bots et al. 2008, Naqvi and Lee 2014). CIMT is one of the most established subclinical biomarkers of atherosclerosis in population based studies, as well as in the domain of occupational and environmental health settings (Hoffmann 2015).

In contrast to the substantial evidence that a onetime measure of CIMT predicts events independent of CVD risk factors, the evidence for progression of CIMT is currently less convincing. Although a recent meta-analysis did not present conclusive evidence that progression of CIMT can predict CVD events (Lorenz, Polak et al. 2012), some researchers continue to report that change in CIMT can be a predictor, especially in high risk populations. In addition, certain limitations of the meta-analysis, including heterogeneity of the populations and the carotid scan protocols, and whether the measurement of CIMT in the segments included plaque or not are worth noting. Moreover, many others reported a positive association. Furthermore, the IMPROVE study found that the greatest progression of maximum CIMT in any carotid segment predicted first CVD events (Baldassarre, Veglia et al. 2013). The MESA study also reported that progression of CIMT can predict the incidence of stroke (Polak, Pencina et al. 2011). This association was also shown in other populations (Okayama, Mita et al. 2013).

CIMT measured via B-mode ultrasound reflects the carotid artery wall thickness captured by imaging and measuring the distance between the intimal-luminal and medial-adventitial interfaces. Although varied protocols are listed in epidemiologic studies, typical scanning protocols include both the left and right carotid arteries, and include multiple carotid artery segments (common carotid artery (CCA), bulb, and internal carotid artery (ICA)) (Stein, Korcarz

et al. 2008). However, there are many population-based and clinical studies that have only measured CCA IMT. The measurement of CIMT across all carotid segments may be a better measure of atherosclerosis burden, as carotid artery thickening and plaque development are more likely to occur in segments exposed to greater turbulent blood flow and sheer stress such as the bulb and ICA (Galbusera, Zoja et al. 1997, Zaman, Helft et al. 2000). Furthermore, Peters et al. recommended that using maximum versus mean CIMT of the all segments would be a better biomarker of atherogenic status because it is more likely to capture atherosclerosis lesion development than present (Peters and Bots 2013).

Plaque, a biomarker and more direct measure of atherosclerosis can also be assessed by B-mode ultrasound. Plaque appears to be a better biomarker to predict CVD risk compared to CIMT alone (Inaba, Chen et al. 2012). Quantitative measures of plaque are an even better predictor (Naqvi and Lee 2014). However, limited studies have considered plaque progression. Two investigations showed that progression of carotid plaque volume could predict future CVD events (Wannarong, Parraga et al. 2013, van Engelen, Wannarong et al. 2014), and another considered plaque area as a predictor (Spence, Eliasziw et al. 2002). However, these three studies were conducted among patients who already had significant atherosclerosis. One community study conducted in Taiwan observed that progression of plaque scores can predict CVD in an unadjusted model and a model adjusted for age and sex, but not in the models considering traditional CVD risk factors (Chen, Jeng et al. 2016).

1.4 SHORT-TERM AIR POLLUTION AND CVD EMERGENCY ROOM VISITS

The acute effect of air pollution and cardiovascular health has drawn quite a bit of public attention since the Great Smog in London in 1952 (Urbinato 1994). After this catastrophic event, laws and policies were implemented to protect the public's health. Since the year 2000, there are an increasing number of epidemiological studies exploring the association between air pollution and CVD morbidity and mortality (Koken, Piver et al. 2003, Bell, Dominici et al. 2005, Ballester, Rodriguez et al. 2006, Dominici, Peng et al. 2006, Ito, Mathes et al. 2011, Tian, Li et al. 2012, Breitner, Wolf et al. 2014, Dai, Zanobetti et al. 2014, Talbott, Rager et al. 2014, Alessandrini, Stafoggia et al. 2016). Most of these studies have established a positive association between air pollution and CVD outcomes.

With the improvement of emergency response, death from CVD within 24-hour admission to hospital has decreased dramatically (Chambless, Keil et al. 1997). Thus, this review is going to use the CVD emergency department visits as the primary outcome of interest. There are two ways to explore the acute effect of air pollution: case crossover and time series analysis. Case crossover studies match the people with themselves before or after one or two weeks on the same weekday of the exposure. Thus, the confounders, like age, race, gender, socioeconomic status (SES) and unobserved factors are controlled in the analysis. Because of this we can stratify to determine if there are effect modifiers from the available data, with the limitations that we cannot include the full sample in one single model. Time series analysis, on the other hand, explores the short-term effect of exposure and outcome, but it also can account for other confounders, like trend over time, spatial autocorrelation, and other potential confounders. Most of the current published studies have taken place in one or several metropolitan areas, the air pollution assessed was regional, and the socioeconomic factors between the neighborhoods are

usually correlated, so time series is an appropriate method to use to explore these factors. However, the health outcomes data collection for this type of studies was usually from public or government sources. For instance, the mortality data were from death certificates, and the hospitalization data were from the government registry or insurance claims (Bell, Dominici et al. 2005, Peng, Bell et al. 2009, Ito, Mathes et al. 2011). Furthermore, CVD hospital admissions are more prevalent among people 65 years and older than ED visits are (National Center for Health Statistics 2011). Therefore, it may be an outcome which can reflect the effect of air pollution in this population, not including the younger population who also suffer from CVD due to air pollution. Winquist et al. showed that the effect size did not change significantly differences between ED visits and hospital admissions among the non-fatal CVD events (Winquist, Klein et al. 2012). However, for fatal outcomes, ED visits may be a better source for outcomes of death. Hospital admissions may present less severe events (Winquist, Klein et al. 2012), since some of these visits may be scheduled or not as severe as cardiac arrest which need immediate assistance. This review is going to examine the association between $PM_{2.5}$ and CVD emergency visits. I hypothesis that on the days of higher $PM_{2.5}$ exposure and shortly after, the local emergency room will have more CVD events compared to lower exposure days.

1.4.1 Current Literature on Air Pollution and CVD Emergency Room Visits

Table 1-1 summarized the current literature on air pollution and CVD emergency room visits. We also included the results from PM_{10}. Unlike $PM_{2.5}$, PM_{10} cannot penetrate very deep into the lungs. The reason why PM_{10} was also included in this literature review is that the correlation between two pollutants is very high, ranging from 0.7 – 0.9 (Roemer and van Wijnen 2001). The monitoring of PM_{10} is only available in earlier years. Most places began to monitor $PM_{2.5}$ in

1999 in the U.S. (U.S. Environmental Protection Agency 2016). The inclusion of the papers about PM_{10} can provide more evidence on the relationship between PM and acute outcomes in CVD, and may also illustrate some earlier findings of high concentrations of PM. Also, in some countries, $PM_{2.5}$ measurements might not be available until very recent years (Pun, Yu et al. 2014). Thus, it could help us to include the information among different population groups.

Most of the current literature illustrates a positive association between $PM_{2.5}$ and acute CVD outcomes on the same day or one day before the ED visits or hospital admissions (Metzger, Tolbert et al. 2004, Ito, Mathes et al. 2011, Liu, Breitner et al. 2013, Sarnat, Winquist et al. 2015). Most of the studies adjusted for several potential confounders, including temperature, or apparent temperature, day of the week, holidays, seasonal effect and long-term trend. Interestingly, none of these studies adjusted for age and sex, which were usually available from the data sources they used. This illustrates that the response for ED visits and CVD is very rapid. However, the effect size is rather small. Most of the papers showed an effect size of less than a 10% increase in ED visits due to each inter-quartile range (IQR) increase in $PM_{2.5}$. This may be due to the limited information from the data source, like local health department records, emergency calls, discharge forms or Medicare claims. Thus, we cannot account for other potential confounders, e.g. BMI, smoking and comorbidities.

Studies examining specific source or air pollution events may be more applicable for policy implication. Sarnat and colleagues analyzed the specific sources of $PM_{2.5}$ and tracers of these sources in the metropolitan Atlanta area, US (Sarnat, Marmur et al. 2008). They found that per IQR increase of $PM_{2.5}$ from gasoline, diesel and biomass burning was significantly related to a 1.5% - 3.5% increase in CVD ED visits. Unlike the studies conducted in the whole study period, Kashima et al. and Barnett et al. included the dust storms in their analysis and found a

much bigger effect size, about 30% increase at the time of the event (Barnett, Fraser et al. 2012, Kashima, Yorifuji et al. 2014). This implied that at the extremely high exposure to air pollution, the effect of air pollution on CVD was very significant. Asian dust from China and Mongolia blew to Japan, causing the level of suspended PM increase; and thus, increased the CVD and cerebrovascular ambulance calls by about 30% during severe dust days (Kashima, Yorifuji et al. 2014). On the contrary, a sand storm in Australia during September 2009 did not showed any statistically significant association by PM during the event, but on the first day of the storm the ED visits increased by 39% (Barnett, Fraser et al. 2012). Another study of the vegetation fire in Darwin, Australia did not find any statistically significant association between PM_{10} and CVD and ischemic heart disease (IHD) ED visits during the dry season, when vegetation fire took place (Hanigan, Johnston et al. 2008).

Most of the investigations considered up to three-day lags in their models and most of them were able to find a statistical significant association between $PM_{2.5}$ and CVD ED visits. This implied that air pollution triggered the CVD event within a very short period of time. A Beijing investigation by Liu and colleagues assessed a wide range of particle sizes and showed that ultrafine particles gave a delayed effect (lag 4 – lag 10), and fine particles has an immediate effect (2-day moving average) (Liu, Breitner et al. 2013). The definition of $PM_{2.5}$ includes all the particles sized less than 2.5 μm, which also includs the ultrafine particles. When an area or environment has a significant concentration of ultrafine particles, these particles can condense and coagulate into bigger particles within a fine particle range in about one day. So, the possible delayed effect may reflect the period within which particle size changes to 2.5 μm range.

$PM_{2.5}$ chemical components analysis can help delineate which specific constituent may be most deleterious to health. There are only seven papers so far that analyzed the association

between PM chemical components and CVD ED visits. A study in Hong Kong by Pun et al. analyzed the chemical components of PM_{10}, and found in one-pollutant models that EC, OC, and nitrate were related to CVD ED visits, and in multi-pollutant models that nitrate, sodium and chloride were significantly associated to the outcome (Pun, Yu et al. 2014). However, each of these chemical components only contributes to CVD ED visits by less than 2%. The remaining five papers all considered the components of $PM_{2.5}$. The study in London, England conducted by Atkinson et al. did not find any significant results for nitrate, sulfate and chloride related to CVD admissions (Atkinson, Fuller et al. 2010). The rest of the four U.S. studies showed a statistically significant association between EC and Zn related to CVD ED visits (Ito, Mathes et al. 2011, Boehm Vock, Reich et al. 2015, Sarnat, Winquist et al. 2015). The findings for other chemicals were various. Sarnat et al. found that hopanes ($C_{30}H_{52}$) were related to CVD ED visits in the St. Louis study, it was the only study that included VOCs in the analysis (Sarnat, Winquist et al. 2015). Ito et al. found more chemicals related to CVD acute admissions, that OC, SO_4, Se and Vr were also contribute to CVD ED visits (Ito, Mathes et al. 2011). Sarnat and colleagues conducted another study in Atlanta, GA, and using the components to identify the source of the chemicals, they found that gasoline, diesel, wood smoke, metal processing source, and Zn, EC, potassium, and OC tracers were all related to CVD ED visits (Sarnat, Marmur et al. 2008). Peng et al. using the Medicare claims of ED visits found that EC, NO_3^-, OCM and NH_4^+ were related to CVD ED visits in the single pollutant model, but only EC remained significant in the multi-pollutant models (Peng, Bell et al. 2009). In an earlier study with a rather short period of PM2.5 data availability, Metzger et al. found the effect of EC, OC and oxygenated hydrocarbon at lag 0 were related to CVD ED visits. However, none of these papers address the potential multi-comparison

issues in the single-pollutant models; also, does not address model mis-specification. And only one paper showed the results of multi-pollutant models (Peng, Bell et al. 2009).

The chemical components were mostly based on EC, OC, nitrate, sulfate and ammonia. Thus, the measurement of all the other components may be very difficult, since they make up less than 1% of the total mass of $PM_{2.5}$. Also, the concentration of these chemicals was rather low, and perhaps too low to make a difference in health. Sarnat et al. also included the VOCs in their analysis and found Hopanes (a polycyclic compound) significantly related to congestive heart failure (Sarnat, Winquist et al. 2015). VOCs are a group of volatile chemicals, and the amount on the filter reduces rapidly. Therefore, the measures of these chemicals may not be very accurate. Moreover, the studies did not address whether they used the single- or multi- pollutant models. If they used a single-pollutant model, they did not address the multi-comparison issues. This implies that the positive findings may be in reality, false positives. The study in Atlanta, GA focused on urban sources of the PM, and used some PM components as tracers which indicated sources that were also found to have significant association with EC (Sarnat, Marmur et al. 2008). But, the major findings indicated that diesel, gasoline, wood combustion and metal processes may be important contributors to PM components related to adverse health outcomes. Since PM is a very complex pollutant and there are challenges in analyzing all the components due to the small proportion of some chemicals and the volatility of some components. Therefore, the sources of this complex mixture would help us to better understand which anthropogenic activities related most to health outcomes. Also, the identification of harmful sources would help EPA to implement policies and regulations to protect human health.

Only one paper addressed spatial dependence (Boehm Vock, Reich et al. 2015), this was a methods paper comparing different spatial models to address the various sources and chemical

components of $PM_{2.5}$. However, the 115 counties selected by this study were scattered all over the U.S. and no spatial autocorrelation parameters were presented. Furthermore, they did not show any spatial autocorrelation statistics in the paper to assess the spatial. Moreover, they did not report the results from non-spatial dependent models, to show the differences between two models. The spatial dependent of studies in the Metropolitan area may be necessary, since the exposure was regional and a lot of unobserved factors were also spatially clustered, like socioeconomic factors. But, this is not the case in this paper. Not accounting for spatial variance or clustering in these papers may due to several reasons. One possible reason for this is that these investigations did not have enough spatial variance in the exposure assessment. Most papers used data from one metropolitan area, and used the monitor data directly without any modelling. Thus, with limited numbers of monitors, the differences in the exposure estimates based on the monitors alone were reduced. The uncertainty of the measurements varied largely. This is especially true in urban areas, where the traffic density, emissions from superfund sites, and meteorology may cause exposure to vary from one place to another even though the two places may not be very far apart (Tunno, Michanowicz et al. 2015). During a temperature inversion, which usually results in a high concentration of ground-level air pollutants, the differences from place to place may be more significant. A study in New York City showed that using the spatiotemporal modeled air pollutants the exposure level have a stronger association with asthma outcomes than the usual temporal modeled exposure (Shmool, Kinnee et al. 2016). Thus, there was low contrast between individual level exposure estimation. This could be another reason why the effect sizes of most of the papers were very small.

1.4.2 Potential Effect Modifiers

1.4.2.1 Gender

Males and females may respond differently to air pollution. Pope et al found that $PM_{2.5}$ was related to vascular function among females, but not males (Pope, Hansen et al. 2011). Bell et al found that among females, the impact of $PM_{2.5}$ is larger on arrhythmias (AR) in the U.S. population national wide, as well as in the northeast of the U.S. (Bell, Son et al. 2015). Carlson et al. also observed a larger effect of O_3 on all-cause ED visits among females compared to males (Carlsen, Forsberg et al. 2013). However, this gender difference is not consistent across studies. Pope and colleagues also found that the association between $PM_{2.5}$ and AMI was larger among males than among females in two other studies (Pope, Muhlestein et al. 2006, Pope, Muhlestein et al. 2015). On the other hand, Rodopoulou et al. only noted the association between $PM_{2.5}$ and CVD emergency room visits among males in the cold period of the year, not among females, in central Kansas (Rodopoulou, Samoli et al. 2015). Also, some studies did not observe gender differences between short-term air pollution exposure and CVD outcomes (Haley, Talbot et al. 2009, Bunch, Horne et al. 2011, Kloog, Nordio et al. 2014). In a meta-analysis, Bell et al. did not discover a stronger association between air pollution and total mortality among females, as the elevated effect size was very small (Bell, Zanobetti et al. 2013). Findings to date are not conclusive regarding gender as an effect modifier in the air pollution and CVD relationship.

1.4.2.2 Race

The racial differences in the effect of air pollution and CVD mortality and morbidity has been less examined (Bell, Zanobetti et al. 2013). Some studies have suggested that racial differences exist with different exposures on outcomes. (Rodopoulou, Samoli et al. 2015, Wing, Adar et al.

2015, Montresor-Lopez, Yanosky et al. 2016). Rodopoulou et al. found that $PM_{2.5}$ and O_3 had a larger impact on CVD emergency room visits among White, in central Arkansas, where they have more Black cases than White. On the other hand, Wing et al. found that $PM_{2.5}$ had a larger effect on ischemic stroke among non-Hispanic White; while O_3 has a greater association with ischemic stroke with the Mexican American, in the Brain Attack Surveillance in Corpus Christi (BASIC) project (Wing, Adar et al. 2015). However, Montresor-Lopez et al. did not observe any significant association between O_3 exposure and stroke in the overall population, or any racial groups, and the effects between the two racial groups were very close (Montresor-Lopez, Yanosky et al. 2016). Because there is very limited evidence for racial differences, in our study we will examine races as a potential effect modifier. As there are usually disparities of race in terms of CVD risk factors, (e.g. residential type, living close to the emission sources, and genetic predisposition) (Glad, Brink et al. 2012). This will be an important effect modifier to consider.

1.4.2.3 Age

In most studies, they observed a stronger effect of air pollution on CVD outcomes in older populations (Pope, Muhlestein et al. 2006, Haley, Talbot et al. 2009, Pope, Muhlestein et al. 2015). In the meta-analysis by Bell and colleagues, they found a stronger relationship between air pollution and mortality among the older population than in the younger population (Bell, Zanobetti et al. 2013). During the aging process, the risk to CVD increases, and the strength of the immune system and metabolic rate decreases; and thus, the elderly are more susceptible to air pollution (Veronica and Esther 2012).

1.4.2.4 Season/temperature

There are several reasons that the season or temperature may be an effect modifier of air pollution and CVD outcomes: (1) studies show that people usually have more outdoor activities in the warm seasons than winter, so the behavior changes based on season (Tucker and Gilliland 2007); (2) the photochemical O_3 relies on precursors (NOx and VOCs) and ultraviolet, which also varies by season (U.S. Environmental Protection Agency 2015); (3) the heating system is used during the winter, so more energy is consumed, which may generate more pollutants and different components of the pollutants in the atmosphere (Bell, Ebisu et al. 2008). Several studies have found a bigger effect of air pollution during the cold season (Ito, Mathes et al. 2011, Talbott, Rager et al. 2014). Moreover, a study in Shanghai observed the effect of SO_2, NO_2 and O_3 on CVD mortality during the cold season only (Kan, London et al. 2008). However, there are some varied findings. Although Ito et al noticed a larger effect on CVD hospitalization during the cold season, they found a larger effect of $PM_{2.5}$ on CVD mortality during the warm season (Ito, Mathes et al. 2011).

Also, extreme temperature can be a risk factor for CVD events. This has been explored in several studies, and all of them revealed an association between temperature and CVD morbidity or mortality (Bhaskaran, Hajat et al. 2009, Tian, Li et al. 2012, Breitner, Wolf et al. 2014, Lavigne, Gasparrini et al. 2014, Zhang, Li et al. 2014, Lian, Ruan et al. 2015). However, a few other studies only observed the cold effect on health (Bhaskaran, Hajat et al. 2010, Claeys, Coenen et al. 2015, Hsu, Hwang et al. 2017). One possible explanation for this is that these studies were conducted in different regions of the world, and thus, with a different latitude and temperature over the year. Xiao et al conducted a study in 13 eastern cities in the U.S. and revealed that in the north, the hot effect had a larger impact on mortality, and in the south, the

cold effect had a bigger influence on mortality (Xiao, Peng et al. 2015). Also, another study conducted in the China had the same conclusion when using stroke as an outcome (Zhang, Li et al. 2014).

1.4.3 Comorbidities

People with comorbidities are more susceptible to air pollution exposure. Alessandrini et al found that people with two or more acute conditions or with some chronic conditions were at a higher risk of CVD death related to short-term $PM_{2.5}$ exposure in a 12 cities study in Italy (Alessandrini, Stafoggia et al. 2016). Also, Peel et al also found that people with comorbidities, especially with hypertension, diabetes and chronic obstructive pulmonary disease were more susceptible to air pollution in terms of CVD emergency room visits (Peel, Metzger et al. 2007). However, as most of the data sources did not provide the information of the other morbidity, so it is not well studied for the acute effect of air pollution on CVD outcomes.

1.4.4 Limitations and Gaps

There are studies utilizing emergency room visits as the outcome, and among these studies, some of them only use a single source (Linares and Diaz 2010, Barnett, Fraser et al. 2012, Carlsen, Forsberg et al. 2013, Liu, Breitner et al. 2013, Rodopoulou, Chalbot et al. 2014), or without a definite diagnosis (Kashima, Yorifuji et al. 2014, Zauli Sajani, Alessandrini et al. 2014). Moreover, none of these studies had any spatial variation in exposure assessment, and only accounted for the temporal effect of the air pollution exposure. Therefore, future studies with the exposure modeling accounting for the spatial variation and meteorological factors are needed.

Furthermore, data from multiple hospitals, which may have a better coverage of the study area, would help to select a study population that is more representative and have greater statistical power.

1.4.5 Conclusion

In conclusion, there is a positive association between $PM_{2.5}$ and acute CVD events. Among the chemical components, EC is the most likely one related to the CVD ED visits. In urban areas, PM from gasoline, diesel and biomass burning and metal process were the most harmful sources to health. Reducing these sources in urban areas, and removing such industry from populated areas can protect human health. However, the current literature only accounted for the temporal effect of air pollution and not spatial variation of exposure. Moreover, all of these papers used time-series analyses, but we will apply a case-crossover design with spatiotemporal exposure estimation for all the air pollutants at the ZIP code level. As ZIP code areas are much smaller than counties, there are a lot of zeroes assigned to this small unit. Thus, the sample is not follow a Poisson distribution. Also, if spatiotemporal exposure was adopted to the model, we will need to set up spatial dependence in addition to the time-series, which will complicate the model. To simplify the statistical analysis, a case-crossover design can observe the temporal effect of these air pollutants with spatial variation of exposure.

1.5 SUMMARY TABLE

Table 1-1 Current Studies of Short-term Air Pollution and Cardiovascular Emergency Department Visit

Author, Year	Population	Study Period	Source	Site	Exposure	Lag	Findings
$PM_{2.5}$							
Sarnat, 2015	1,733,543 total visits, 69,679 for CVD, and 186,449 for RD disease	06/01/2001-04/30/2013	Billing records from Missouri Hospital Association	St. Louis Metropolitan area (36 out of 43 hospitals)	$PM_{2.5}$ Sulfate Nitrate OC, EC, Si, K, Ca, Fe, Cu, Zn, Pb, some VOCs	Lag 0-2 Total impact	EC and OC was signficiantly related to CVD and CHF. Hopanes was related to CVD, and Zn contributed to CHF.
Kashima, 2014	51,945 elderly (≥65 yr)	Jan 2006 – Dec 2010	Ambulance Division of Cities fire Bureau	Okayama, Japan	Asian dust SPM	lag 0 to lag 4	Asian dust and SPM were related to all cause, CVD and cerebrovascular. In the two-pollutant model, asian dust was associated with all cause, CVD and pulmonary disease at lag 3, cerebrovascular at mean exposure of lags 0-4.
Boehm Vock, 2014 (method paper)	115 counties	2000-2008	Medicare claims	115 counties in the US	$PM_{2.5}$ components		EC significantly associated with CVD ED visits in spatial model, exchangeable variable selection (VS) model, and spatial VS model
Rodopoulou, 2015	84,269 CVD and 29,402 RD	2002–2012	UAMS Medical Center, only state-owned medical serves uninsured and Medicare/Medicaid patients in Arkansas	Central Arkansas	$PM_{2.5}$ O_3	Lag 0 to lag 2	No statistical significant association was found. The effects of $PM_{2.5}$ were stronger among whites, but for the respiratory effects of O_3 that were higher among Blacks/African-Americans (NS).
Rodopoulou, 2014	4739 RD and 2031 CVD ED visits 46.7% Hispanic, and 16.7% Latino	2007-2011	ED visits and hospital admissions from the Memorial Medical Center	Dona Ana County, New Mexico	$PM_{2.5}$ $PM_{2.5-10}$ PM_{10} O_3	Lag 1	No significant association was found
Liu, 2013	13,026 CVD ED visits	03/04/2004 – 12/31/2006	Standard medical record forms from the ED of Peking University's	Beijing, China	Ultrafine PM $PM_{2.5}$ $PM_{2.5-10}$	Lag 0-lag 10 2-day	Number concentration of ultrafine PM was associated with CVD ED visits at lag4 – lag10, also with 2-

Table 1-1 Continued

Author, Year	Population	Study Period	Source	Site	Exposure	Lag	Findings
			Third Hospital			and 11-day moving average	day and 11-day moving average. 2-day moving average of $PM_{2.5}$ mass concentration was associated with all-cause ED visits, but no longer significant with severe CVD ED visits.
Winquist, 2012	5,709,926 ED visits and 1,999,708 HA records, 1,024,228 HA through ED	01/01/2001 - 06/27/2007	Missouri Hospital Association for all ED visits and 28 of 29 acute care hospitals	St. Louis Metropolitan area	$PM_{2.5}$ O_3	Lag 0-lag 4 total impact	$PM_{2.5}$ was related to Asthma/wheeze ED visits, and stayed significant in 2-18 yr group, but not related to poverty area. O_3 was related to asthma/wheeze and CHF in all visits types, in 2-18 yr group; and related to CVD and CHF ED visits in poverty area.
Barnett, 2012	9,098 before the storm and 1,353 after the storm	01/01/2009 - 10/31/2009	Emergency admissions at Prince Charles Hospital	Brisbane, Australia	$PM_{2.5}$ PM_{10} Dust storm	Lag 0 – lag 21	No increased association was found ED increased 39% at the first day of the storm
Ito, 2011	281.3 CVD ED visits per day	2000-2006	Hospitalization and mortality at the New York City Department of Health and Mental Hygiene	New York City, NY	$PM_{2.5}$ $PM_{2.5}$ components NO_2, SO_2, CO	Lag 0 – lag 3	EC, SO_4, Si, Se and Br were associated with CVD mortality at lag 1. $PM_{2.5}$, EC, OC, SO_4, Zn, Se, Vr, NO_3, NO_2, SO_2, and CO were related to CVD hospitalization at lag0.
Mathes, 2011	580,841 syndrome ED visits (44,427 CVD)	01/01/2000-06/30/2002 (11 of 50 hospitals) 01/01/2004-12/21/2006 (50 hospitals)	ED chief complaint and discharge at the New York City Department of Health and Mental Hygiene	New York City, NY	$PM_{2.5}$	Lag 0 – lag 3	$PM_{2.5}$ was significantly associated with CVD hospitalization, cardiac ED visits and CVD ED visits at lag0 in cold season, but not in warm season or all year.
Atkinson, 2010	153 median daily admissions in CVD	01/01/2000 – 12/31/2005	All admissions from National Health Service hospitals in England	London, England	PM2.5, PM10 Black smoke PM components	Lag 0 – lag 6	$GRPM_{10}$ and $GRPM_{2.5}$ were related to CVD hospital admissions at lag 2, and lag 2 and lag 3, respectively. No other measures of PM_{10} and $PM_{2.5}$ found any significant

Table 1-1 Continued

Author, Year	Population	Study Period	Source	Site	Exposure	Lag	Findings
							association.
Linares, 2010	23,016 emergency admissions ≥75 years 7672 CVD, and 6357 RD	01/01/2003-12/31/2005	Emergency hospital admissions at the Gregorio Maranon University Teaching Hospital	Madrid, Spain	$PM_{2.5}$ PM_{10} Temperature NO_2, O_3, SO_2, NOx	Lag 0 – lag 3?	$PM_{2.5}$ related to CVD and all cause at lag0, and RD at lag3 in whole year The association remained in winter, but not in summer
Peng, 2009	Not reported	2000 - 2006	Medicare claim data with emergency or acute in the database	119 counties in the U.S.	$PM_{2.5}$ SO_4^{2-}, NO_3^-, OCM, EC, Si, Na^+, NH_4^+	Lag 0 – lag 2	$PM_{2.5}$ was significantly associated with CVD ED visits. EC was significantly related to CVD ED visits in both single and multi- pollutant model. But, NO_3^-, OCM and NH_4^+ was only significantly related to outcome in the single pollutant model.
Sarnat, 2008	>4.5 million ED visits. 324 CVD per day, and 75 RD per day on average.	Nov 1998 – Dec 2002	37 out of 45 hospitals in the metropolitan Atlanta Area	Metropolitan Atlanta Area, 20 counties	$PM_{2.5}$, and $PM_{2.5}$ by source and tracer	Lag 0	$PM_{2.5}$ was associated with all CVD ED visits; the association remained in gasoline, diesel, wood smoke, metal processing source, and Zn, EC, potassium, and OC tracers.
Metzger, 2004	4,407,535 CVD events	01/01/1993 – 08/31/2000 ($PM_{2.5}$ after 08/01/1998)	31 hospitals in the metropolitan Atlanta Area	Metropolitan Atlanta Area, 20 counties	PM_{10}, O_3, NO_2, SO_2, CO, $PM_{2.5}$ and $PM_{2.5}$ components	3-day moving average, Lag 0 – lag 7	3-day moving average of NO_2, CO, SO_2 and $PM_{2.5}$, OC, EC and oxygenated hydrocarbon were related to all CVD ED visits. $PM_{2.5}$, NO_2, and CO at lag 0 and lag 1 were associated with all CVD visits; and OC, EC and oxygenated hydrocarbon at lag 0 were related to CVD ED visits.
PM_{10}							
Pun, 2014	400,011 CVD and 587,422 RD ED visits	01/01/2001 - 12/31/2007	Hong Kong Hospital Authority daily counts of ED admissions of public funded hospital (90%)	Hong Kong	PM_{10} components	Lag 0 – lag 3 2-day moving average	PM_{10}, EC, OM and NO_3^- were related to CVD at lag0, lag2 and lag3. The same association was also found in the 2-day MA models

Table 1-1 Continued

Author, Year	Population	Study Period	Source	Site	Exposure	Lag	Findings
						(lag 0 and lag 1)	The multi-pollutant model was only found NO_3-, Na+ and Cl- significantly associated with CVD ED visits
Rodopoulou, 2014	4739 RD and 2031 CVD ED visits 46.7% HIspanic, and 16.7% Latino	2007-2011	ED visits and hospital admissions from the Memorial Medical Center	Dona Ana County, New Mexico	$PM_{2.5}$ $PM_{2.5-10}$ PM_{10} O_3	Lag 1	When excluding extreme PM days, PM10 is associated with RD ED visits.
Zauli Sajani, 2014	Daily mean EAD of people aged 35-year and older is 201, in which 12.5% were CVD, and 8.9% were RD	2002-2006	Emergency ambulance dispatches (EAD)	6 towns in Emilia-Romagna, Italy	PM_{10}	Lag 0-lag 1	The association between PM10 and EAD of CVD and RD was not significant, but significant with hospital admissions and mortality. There was a significant association between PM10 and CVD and RD EAD in warm period.
Carlsen, 2013	24439 ED visits of ≥18 years, 18782 of CVD, 4082 of stroke, and 1575 of pulmonary	Jan 2003 – Dec 2009	ER visits and acute hospital admissions to the Landspitali University Hospital (only acute care hospital in the area)	Reykjavik, Iceland	PM_{10} O_3 NO_2	Lag 1 Mean of lag 0 – lag 2	O_3 is related to all cause ED visits. There was no association with PM_{10} or NO_2 was found. The association between O_3 and ED were higher among the females and slightly higher among the elderly.
Barnett, 2012	9,098 before the storm and 1,353 after the storm	01/01/2009 - 10/31/2009	Emergency admissions at Prince Charles Hospital	Brisbane, Australia	$PM_{2.5}$ PM_{10} Dust storm	Lag 0-lag 21	No increased association was found ED increased 39% at the first day of the storm
Atkinson, 2010	153 median daily admissions in CVD	01/01/2000 – 12/31/2005	All admissions from National Health Service hospitals in England	London, England	$PM_{2.5}$, PM_{10} Black smoke PM components	Lag 0 – lag 6	$GRPM_{10}$ and $GRPM_{2.5}$ were related to CVD hospital admissions at lag2, and lag2 and lag3, respectively. No other measures of PM_{10} and $PM_{2.5}$ found any significant association.
Linares, 2010	23,016	01/01/2003-	Emergency hospital	Madrid,	$PM_{2.5}$	Lag 0 –	PM_{10} was not associated with any

Table 1-1 Continued

Author, Year	Population	Study Period	Source	Site	Exposure	Lag	Findings
	emergency admissions ≥75 years 7672 CVD, and 6357 RD	12/31/2005	admissions at the Gregorio Maranon University Teaching Hospital (reference)	Spain	PM_{10} Temperature NO_2, O_3, SO_2, NOx	lag 3?	outcomes.
Buadong, 2009	33,458 total CVD visits, 1876 arrhythmia, 2566 MI and 10158 IHD	04/01/2002 – 12/31/2006	Three government hospitals	Bangkok, Thailand	PM_{10}, O_3	Lag 0, lag 1 and mean of 2 days and 3 days	PM_{10} and O_3 were related to total CVD at lag 1 and 2 day mean exposure among the people who 65 years and older. O_3 was also related to total CVD in the total population at lag 1.
Hanigan, 2008	8,729 ED admissions of CVD or RD	04/01/1996 – 11/30/2005 dry season	The Royal Darwin Hospital provided by the Northern Territory Department of Health and Community Services	Darwin, Australia	Vegetation fire, PM_{10}	Lag 0 – lag 3	No association were found between PM_{10} and outcomes among the non-indigenous or indigenous population.
Filho, 2008	45,000 ER visits for CVD, and 7,000 were diabetic	Jan 2001 – July 2003	Secondary health data from the Sao Paulo Hospital of the Medical School of the Federal University of Sao Paulo	Sao Paulo, Brazil	PM_{10}, O_3, CO, SO_2, and NO_2	Lag 0 – lag 3 2 – 4 days moving average	No significant association was found between PM_{10}, O_3 and CVD ER visits among diabetic or non-diabetic patients. Some significant findings with CO, NO_2, and SO_2 were noted.
Ulirsch, 2006		Nov 1994 – Mar 2000	Admissions and medical visits (not distinguish the scheduled and unscheduled ones)	Pacatello and Chubbuck, Idaho	PM_{10}, SO_2 and NO_2	Lag 0 – lag 4	No significant association was found between PM_{10} and CVD, but with RD.
Ren, 2006	18 RD ED visits per day, and 17 CVD ED visits per day on average.	01/01/1996 – 12/31/2001	Queensland Department of Health	Brisbane, Australia	PM_{10}, temperature	Lag 0 – lag 2	There are interaction between PM_{10} and temperature on CVD ED visits at lag1 and lag2.
Metzger, 2004	4,407,535 cases	01/01/1993 – 08/31/2000	31 hospitals in the metropolitan Atlanta	Metropolitan Atlanta Area,	PM_{10}, O_3, NO_2, SO_2,	3-day moving	PM10 at lag 0 were related to all CVD ED visits.

Table 1-1 Continued

Author, Year	Population	Study Period	Source	Site	Exposure	Lag	Findings
		(PM2.5 after 08/01/1998)	Area	20 counties	CO, $PM_{2.5}$ and $PM_{2.5}$ components	average, Lag 0 – lag 7	

Notes: PM: particulate matters; SPM: suspended particulate matters; GRPM: gravimetric particulate matters; EC: elementary carbon; OC: organic carbon; OM: organic materials
CVD: cardiovascular disease; CHF: congestive heart failure; RD: respiratory disease; ED: emergency department
Lag: days before the event

1.6 LONG-TERM AIR POLLUTION AND SUBCLINICAL ATHEROSCLEROSIS

Few studies have explored the association between long-term air pollution and CVD (Nogueira 2009, Franchini and Mannucci 2012), and even fewer studies have explored the relationship between long-term air pollution and subclinical atherosclerosis.

In this section, we are going to review the current literature on ambient air pollution and biomarkers of atherosclerosis, focused on CIMT and plaque.

Table 1-2 summarizes the overall study design and major findings of these 15 studies. There were four prospective cohort studies, three retrospective studies, and eight cross-sectional studies identified.

1.6.1 Cross-sectional Findings between One-year Exposure Air Pollution and Atherosclerosis

Several cross-sectional studies explored the possible association between the annual mean exposure of air pollutants prior to the outcome of an ultrasound scan. The ESCAPE study used a meta-analysis approach to combine results of four community cohorts in Europe: 1) IMPROVE-Stockholm in Sweden, 2) KORA in Germany, 3) Heinz Nixdorf Recall (HNR) in Germany, and 4) REGICOR in Spain, which resulted in more than 9,000 individuals (Perez, Wolf et al. 2015). Land use regression (LUR) was used to estimate air pollution exposure level in all sites by implementing monitors in years 2008 and 2009. Overall, there was no significant association

found between any of the air pollutants or traffic intensity and CIMT. The IMPROVE-Stockholm cohort, which included 487 participants, was the only study in this manuscript that found a positive association between traffic load and CIMT. Participants had 5.5% (95% CI: 1.9% – 9.2%) thicker CIMT per 4,000,000 vehicles-meters/day within a 100-meter buffer of the residential address. In addition, some of the cohort included in the study presented some positive findings. One analysis of the HNR study published earlier found that only $PM_{2.5}$ was related to increased CIMT, but not PM_{10} or distance to major roads (Bauer, Moebus et al. 2010). The participant would have about 4.3% thicker CIMT if they were exposed to an interdecile range of 4.2 $\mu g/m^3$ higher $PM_{2.5,}$ even after further adjusting for traffic, hypertension and antihypertensive medication. They also found that participants who were under 60 years old, more obese, ever smoker, not diabetic, not having coronary heart disease, using statin, and full-time employed for the last five years, were having a stronger association between $PM_{2.5}$ and CIMT. It is not surprising that the two studies using the same population had different findings, since the exposure estimations were different. The HNR study estimated the exposure level based on a chemical transport model during the study period, 2000 – 2003; while the ESCAPE study used the standardized LUR model implemented in years 2008 – 2009. The later one may have some exposure uncertainties, since they have to assume that the spatial variations were the same throughout all years. The CIMT measures used as outcomes in these four cohorts in the ESCAPE study were taken during the years 1997 – 2009.

A study among civil servants with an average age of 61 years old lived in Greater London in the U.K. found that PM_{10} and PM_{10} weighted by oxidative potential (OP) were related to CIMT (Tonne, Yanosky et al. 2012). The participants who had a 5.2 $\mu g/m^3$ higher exposure of PM_{10} had a 5.0% (95% CI: 1.9 – 8.3) thicker CIMT, after adjusting for age, gender, smoking,

BMI, season and year; and with a 1.5 m^{-3} higher exposure to PM_{10} weighted by oxidative stress, they had a 1.2% (95% CI: 0.2% – 2.2%) thicker CIMT after adjustment. Interestingly, when they divided the 52-week exposure into 1 – 13 weeks, 14 – 26 weeks, and 27 – 52 weeks prior to the carotid scan date, only the exposure at earliest lag was statistically significantly related to CIMT thickening: participants had a 4.8% (95% CI: 1.4% – 8.3%) thicker CIMT with 10 $\mu g/m^3$ PM_{10} higher exposure. This may imply there is a latency effect of PM exposure on vascular change. While another study conducted among young adults in the Netherlands (N=745) found that there was no association between NO_2, SO_2, $PM_{2.5}$, black smoke, and proximity to traffic and CIMT, but some associations between NO_2 and SO_2 and blood vessel stiffness (pulse wave velocity (PWV) and augmentation index (Ai)) were found in a subgroup of the cohort (N=500) (Lenters, Uiterwaal et al. 2010).

Two cross-sectional studies from MESA Air were conducted, in which they not only explored the association related to CIMT with $PM_{2.5}$, as well as some components of $PM_{2.5}$ (Sun, Kaufman et al. 2013, Kim, Sheppard et al. 2014). Both articles used more than one estimation method to predict $PM_{2.5}$ level and the association did not remain the same based on these different measures. In Kim et al.'s paper, $PM_{2.5}$ and CIMT were associated in the spatiotemporal model, but not in the national spatial model (Kim, Sheppard et al. 2014). In this paper, the spatio-temporal model used 2-week average measurements from the monitors implemented by MESA air study; whereas, the national spatial model based on the measures from EPA monitors. However, sulfur, silicon and organic carbon (OC) were related to CIMT in both exposure estimation models; but elementary carbon (EC) was not associated with CIMT in both models. On the other hand, Sun et al.'s paper reported a significant association between $PM_{2.5}$ and CIMT in all three estimations: nearest monitor, inverse distance weighting (IDW) and city-wide

average, but the effect size varied from 9.6 μm to 15.8 μm per IQR change of $PM_{2.5}$ (Sun, Kaufman et al. 2013).

Kunzli et al. published the first paper examining the cross-sectional association between $PM_{2.5}$ and CIMT in two RCTs among 798 men and women aged 40 – 89 with treatment of vitamin B and E implemented in the study, respectively (Künzli, Jerrett et al. 2005). They discovered a cross-sectional association between $PM_{2.5}$ and CIMT in the unadjusted models. This association only remained statistically significant in three subgroups, people older than 60 years old, females and people who took lipid lowering drugs, after adjusting for sex, age, education and income. Further adjusting for physical activity, passive smoking, alcohol consumption, and multivitamin intake, they only noted that among women aged 60 years and older had a 13.8% (95% CI: 4.0% – 24.5%) thicker CIMT with a 10 $\mu g/m^3$ higher exposure to $PM_{2.5}$. However, they did not construct any model adjusting for the traditional CVD risk factors. This may be because these risk factors are potential mediators, or the sample size was too small to adjust for more confounders. There are 186 women aged 60 years and older, and only 109 people were on lipid-lowering therapy.

Su et al. conducted the only study in Taiwan to investigate the association between air pollutants and CIMT among a community sample of 689 participants aged 35 – 65 years old in Taipei. They used LUR based on the method of ESCAPE study to estimate the exposure level and found that $PM_{2.5}$ absorption ($PM_{2.5}$ measured by light absorption) was related to left side mean and mean maximum CIMT, but not related to any CIMT outcomes (Su, Hwang et al. 2015). The participants who lived in an area within a 10^{-5} m^{-1} $PM_{2.5}$ measured via light absorption higher exposure had a 4.19% (0.38% – 8.00%) thicker left side common carotid

artery CIMT. This group also found that PM_{10}, NO_2 and NO_x were associated with left side or combined maximum CIMT outcomes.

These findings suggested that there are a positive association between air pollution and atherosclerosis. However, the process to develop atherosclerosis is very long (Künzli, Perez et al. 2011), so the cross-sectional studies assumed that the exposure level did not change over time. Evidences linking long-term air pollution and atherosclerosis will be presented in the next section.

1.6.2 Long-term Exposure to Air Pollution and Atherosclerosis

There are three studies assessed the long-term or lifetime exposure of air pollutants and predicted mean CIMT based on a one-time ultrasound scan (Breton, Wang et al. 2012, Rivera, Basagaña et al. 2012, Armijos, Weigel et al. 2015). Armijos *et al.* and Brenton *et al.* studied this effect among children, so the authors were able to estimate their lifetime exposure based on their residential address (Breton, Wang et al. 2012, Armijos, Weigel et al. 2015). The Ecuador study (Armijos, Weigel et al. 2015) found that children aged 6 – 14 years old who lived closer to traffic had a higher mean and maximum CIMT. Another study (Breton, Wang et al. 2012) conducted among college students in Los Angeles, CA, USA was not able to find any statistically significant results related to $PM_{2.5}$, PM_{10} and NO_2. On the other hand, O_3 was statistically significantly associated with mean CIMT. With each 9.3 ppb increased level of exposure to O_3 during their childhood (6 – 12 years), they had 10.1 μm (CI: 1.8 – 18.5) thicker CIMT, and this association remained statistically significant when adjusting for other air pollutants. This may imply a latency effect of air pollution contributed to atherosclerosis, though in this very young population.

Rivera et al. estimated a 10-year exposure of NO_2 using land use regression and traffic proximity related to CIMT and ankle-brachial index (ABI) among the community population aged 32 – 86 years old in the Girona region, Spain (the REGICOR study) (Rivera, Basagaña et al. 2012). NO_2 and traffic proximity were found to be related to CIMT in the unadjusted model, but only traffic proximity remained statistically significantly associated with CIMT in the model adjusted for confounders. There was 4.9% (95% CI: 2.6% – 7.2%) thicker CIMT per 25 $\mu g/m^3$ higher exposure to NO_2, in the unadjusted model or the model only adjusted for sex, but not any other confounders. With 7,200,000 vehicles-meters/day higher traffic load in the 100-meter buffer of the residential address, there was 2.08% (95% CI: 0 – 4.17%) thicker CIMT in the model adjusted for sex, age, sex–age interaction, smoking status, education, and marital status, but no longer statistically significant in the model further adjusted for BMI, HDL, waist circumference, systolic and diastolic blood pressure, physical activities, diet, medication treatment, and census-tract education level. Traffic density in the nearest street (15,000 vehicles/day), on the other hand, was statistically associated with 1.8% increase of CIMT (95% CI: 0.01 – 3.59) in the fully adjusted model. There was no statistically significant association between any of the exposures and low ABI, but associated with high ABI (> 1.3). ABI out of the normal range (0.9 – 1.3) was an indication of peripheral vascular disease.

The long-term association between air pollution and atherosclerosis was established using traffic as an exposure, as the monitor data were not always available throughout the study period, especially for $PM_{2.5}$, which only has available data in the most recent two decades. Using traffic may reflect the exposure related to traffic emissions, such as $PM_{2.5}$, NOx, and VOCs, but not including the emissions from other sources, like factories. Thus, in our study, we will use the

monitored data for $PM_{2.5}$ and O_3 as our exposure assessment to address their effects on atherosclerosis.

1.6.3 Prospective Association between Air Pollution and Atherosclerosis Progression

Investigators have conducted several prospective cohort studies in order to examine air pollutants and CIMT progression. There have been four studies which have modeled air pollution exposure using either a land-use regression, or a spatiotemporal model. In 2010, Kunzli et al. published the first paper regarding $PM_{2.5}$ and CIMT progression using a combined analysis of five randomized control trials (RCTs) (Künzli, Jerrett et al. 2010). The population consisted of men and women aged 30 to 82 in the greater Los Angeles area, where the traffic is very heavy and has a rather high $PM_{2.5}$ exposure. These five RCTs consisted of two among post-menopausal women in trials using estrogen, one among diabetic patients under the treatment of thiazolidinedione, one among men and post-menopausal women with high plasma homocysteine treated with vitamin B, and another one among the population with high LDL under the vitamin E trial. They noted that 10 $\mu g/m^3$ $PM_{2.5}$ was associated with 4.4% (95% CI: 0.4 – 8.4) CIMT progression in the treatment group after an average of 2 – 3 years follow-up. All trials failed to protect against atherosclerosis, since the effect of these drugs may depend on a host factors or some of the medications were pro-atherogenic. Another limitation of this paper is that the exposure assessment was not temporally accurately assigned. The exposure estimation of $PM_{2.5}$ used a kriging model based on monitors to obtain a spatial variation of year 2000 only. However, the outcome ascertainment of some of the studies were at a very early year, like 1996; thus, the estimation may have a very large uncertainty. In this paper, traffic proximity was associated with CIMT progression, and was more pronounced within the population in the lower income and education groups.

The Normative Aging Study cohort, among white veterans at an average age of 76 years old, explored the association between black carbon, which is a major component of $PM_{2.5}$ (Roemer and van Wijnen 2001), and CIMT progression (Wilker, Mittleman et al. 2013). Among these men, the annual mean exposure per interquartile range (IQR) of black carbon (0.26 $\mu g/m^3$) before the baseline was associated with 1.1% (95% CI: 0.4% – 1.7%) increase in CIMT, but was not associated with CIMT progression. In the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air) study (Adar, Sheppard et al. 2013), land-use regression was used to estimate the exposure level and evaluate the indoor and outdoor exposure time by questionnaire. They found that in people with a 2.5 $\mu g/m^3$ increase from baseline $PM_{2.5}$, there was a 3.8 μm (95% CI: 1.2 – 6.4) higher CIMT progression over time within each site, and per 2.5 $\mu g/m^3$ increase in mean follow-up $PM_{2.5}$, the progression of CIMT was 5.0 μm (95% CI: 2.6 – 7.4) higher; moreover, they found that per 1 $\mu g/m^3$ change in $PM_{2.5}$, CIMT progression was 2.8 μm (95% CI: 1.6 – 3.9) higher. However, another paper reported their 10-year follow-up findings did not find any statistically significant association between $PM_{2.5}$ and CIMT progression (Kaufman, Adar et al. 2016). This finding was limited to the participants who had CIMT scans at both baseline and examination 5, not others. The sample size was about 3500, which was only about 70% of the sample size of their 2.5-year follow-up study. The differences in sample size may reflect a selection bias. Moreover, the reading of the images was not blinded between baseline and examination 5. The reader read the images side-by-side, which might produce an artificial effect of progression (Tattersall, Gassett et al. 2014). A newly published abstract from the MESA Air study reported a ten-year follow-up of O_3 exposure and atherosclerosis progression using both CIMT and plaque as outcomes (Wang, Sheppard et al. 2016). With a 1 interquartile rage

(IQR) increase of 3 ppb O_3 per year, there was a 5.6 μm (95% CI: 1.4 – 9.7) increase in CIMT, and 1.2 (95% CI: 1.1 – 1.4) times greater odds of plaque formation.

Finally, the Multicultural Community Health Assessment Trial (M-CHAT), which included about 500 participants in Vancouver, Canada, was a study with a 5 year follow-up (Gan, Allen et al. 2014). No significant associations were found between $PM_{2.5}$, NOx, noise or traffic proximity and CIMT or plaque progression. When stratified by race, the only significant finding was that among the Chinese, people who lived in the area ≤150 m from a highway, or ≤ 50 meters from a major road, had an increase of 1.12 mm^2 (95% CI: 0.21 – 0.23) annual progression of plaque area in this population.

The heterogeneous findings of these studies suggest that more evidence is needed to ascertain the association between air pollution and progression of atherosclerosis.

1.6.4 Potential mechanisms and model establishment

$PM_{2.5}$ is an air pollutant that has been studied extensively with cardiovascular outcomes. In this review, there are eleven articles which studied $PM_{2.5}$, and one which also studied PM_{10}. As discussed before, $PM_{2.5}$ can affect cardiovascular systems through three pathological pathways (Brook 2008, Brook, Rajagopalan et al. 2010, Franklin, Brook et al. 2015). The reviewed papers did not focus on the pathological pathways of air pollution to atherosclerosis, but most papers adjusted for potential intermediate factors (Bauer, Moebus et al. 2010, Lenters, Uiterwaal et al. 2010, Rivera, Basagaña et al. 2012, Adar, Sheppard et al. 2013, Sun, Kaufman et al. 2013, Kim, Sheppard et al. 2014, Kaufman, Adar et al. 2016). However, the effect size was not reduced in these studies. This may imply that there are other pathological pathways of the effect of $PM_{2.5}$ on CIMT. However, we need to keep in mind that additionally adjusting for potential mediators is

not an ideal method to identify mediation. The inflammatory biomarkers in the extended model were C-reactive protein and fibrinogen (Rivera, Basagaña et al. 2012, Adar, Sheppard et al. 2013). These are systemic inflammatory biomarkers. However, PM can also have a direct effect on blood vessels. Furthermore, environmental factors can play a role at every stage leading to CVDs, from risk factors, subclinical and clinical disease and secondary event (Franklin, Brook et al. 2015).

When the risk factors, like blood lipids, obesity, diabetes, inflammation are all affected by PM and lead to subclinical atherosclerosis, it is difficult to correctly address the mediated effect from inflammation by not teasing out the effect of all these other potential confounders, or simultaneous mediators. Thus, the possible linkage through the inflammatory pathway may not be clearly tested in existing epidemiology studies. Another study among young adults did not find any statistical significance with $PM_{2.5}$ after adjusting for blood lipids in the extended model; they were not able to observe any noticeable changes (Lenters, Uiterwaal et al. 2010). There are a few animal studies in which results indicated that the effect of PM on atherosclerosis may be mediated through the lipids pathway (Campen, Lund et al. 2010, Li, Navab et al. 2013). The study in greater London tested PM_{10} and oxidative potential and found the significant associations with CIMT (Tonne, Yanosky et al. 2012). This may imply that the effect of PM might be through an oxidation pathway. No epidemiology study of $PM_{2.5}$ related to oxidative stress has been done. Usually, the increase in oxidative species in the body will lead to higher inflammatory status. Conceptual linear and non-linear associations have been proposed by Araujo and Rosenfeld (Araujo and Rosenfeld 2015). However, in the epidemiological setting, we cannot measure reactive oxidative species (ROS), and cannot test this mechanism directly.

Further studies and hypotheses on biological mechanisms may be proposed to link PM and atherosclerosis.

1.6.5 Limitations and Gaps

The literature to date reflects very heterogeneous findings from these studies, regardless of the design of the study or the location. There are potential limitations of these studies, including exposure misclassification, related to the exposure estimation at a time long before or after the outcomes collection. The ESCAPE study combined several cohort studies to achieve a sufficient sample size, but the CIMT measure study protocols differed across individual studies (Perez, Wolf et al. 2015).

Many of these studies were ancillary studies of the main parent study, which already collected biomarkers of atherosclerosis (Künzli, Jerrett et al. 2010, Tonne, Yanosky et al. 2012, Adar, Sheppard et al. 2013). After the outcome collection, investigators implemented an estimation of air pollutant exposure to the existing study population. So, there were limitations of temporal uncertainties of exposure estimation. First, recall bias might be a concern if the authors want to obtain the residential address information of the participants for a long period (Breton, Wang et al. 2012, Armijos, Weigel et al. 2015). These two studies were not birth cohort studies and they only collected the CIMT measures among children or young adults at one time-point and using the recalled address for their lifetime to estimate the exposure. Furthermore, if the geocoding system changed over time, then the geocoding of the address may not be valid, or not be able to be matched after a decade. Also, in the MESA Air study, the exposure questionnaire about time spent at home, work and outside was collected at or after examination 4, but CIMT scan was collected before then. In their later published paper, they pointed out that there were a

lot of uncertainties in capturing the real exposure level based on the questionnaire (Kaufman, Adar et al. 2016). When people go out may be also very important for the exposure assessment, as the pollution level may be higher during the peak hours. However, the exposure was estimated on a daily basis, and the questionnaire divided day by hours or minutes. Furthermore, a questionnaire cannot capture the behavioral change over time. Moreover, the estimation of air pollution may be limited due to monitor resources, as the LUR monitors were only implemented for two years to address the spatial variations, and the temporal for the ten-year follow-up was derived from the EPA monitors. Thus, in the ten-year follow-up study, they did not establish a robust association between time-weighted $PM_{2.5}$ estimation and progression of CIMT or CAC (Kaufman, Adar et al. 2016). Two cross-sectional studies (Künzli, Jerrett et al. 2005, Lenters, Uiterwaal et al. 2010) used one-year exposure from 2000, but CIMT scan dates varied largely based on the different RCTs. The four cohorts from Europe also estimated the exposure in 2008 and 2009, but the carotid scan was performed between 1997 and 2009 (Perez, Wolf et al. 2015). Some of the cohorts included in the ESCAPE study published their data based on different exposure estimation and reported very different findings. Also, some of the studies used LUR methods to estimate the exposure level, but due to the high cost of installing study monitors at hundreds of sites in the area, they were able to measure only through one year or two, but the follow-up time is much longer (Adar, Sheppard et al. 2013, Peters and Bots 2013). Therefore, the temporal association was not well-established in these studies.

Second, when integrating several studies together to explore the association between air pollutants and CIMT, there were numerous differences in the study protocol. For example, in the ESCAPE study, the HNR study measured CIMT only in a plaque free area, but the other three studies did not follow this protocol, i.e. they may have included plaque in their CIMT measure

(Perez, Wolf et al. 2015). Thus, the measure of CIMT in HNR was much smaller than the other three studies. So, it is not surprising to see the results among the four European cohorts that the statistically significant findings were only in the IMPROVE-Stockholm study. Moreover, different RCTs included in Kunzli et al.'s analysis included populations with varying characteristics, and potential effects from different treatments. (Künzli, Jerrett et al. 2005, Künzli, Jerrett et al. 2010). Therefore, it is very difficult to draw conclusions from these studies without considering the differences and limitations. However, there are some findings which do imply the possible association between air pollutants and CIMT, and some other biomarkers of atherosclerosis.

Eight out of these ten studies found statistically significant associations between $PM_{2.5}$ and CIMT. Three MESA papers found significant associations between $PM_{2.5}$ and CIMT, and one of them found the cumulative exposure of $PM_{2.5}$ was related to CIMT progression (Adar, Sheppard et al. 2013, Sun, Kaufman et al. 2013, Kim, Sheppard et al. 2014). However, the relationship between the long-term $PM_{2.5}$ exposure and CIMT progression was only found in some subgroups when Kunzli combined five RCTs (Künzli, Jerrett et al. 2010). The cross-sectional association between $PM_{2.5}$ and CIMT was only found in some subgroups when published earlier with two RCTs (Künzli, Jerrett et al. 2005). Similar results have been found in other studies, either in the whole cohort, or some subgroups (Bauer, Moebus et al. 2010, Perez, Wolf et al. 2015, Su, Hwang et al. 2015). Two papers that studied PM_{10} as the exposure also found a significant association (Tonne, Yanosky et al. 2012, Su, Hwang et al. 2015).

Although the effect sizes across the studies are different, the positive direction of the association has been consistently repeated from all these studies. Thus, there is an association

between $PM_{2.5}$ and CIMT based on these evidences, which also has been supported by two recently published meta-analyses (Akintoye, Shi et al. 2015, Provost, Madhloum et al. 2015).

1.6.6 Conclusion

There are possible positive associations between air pollutants and CIMT. However, there are limited prospective studies of air pollutants with CIMT and plaque. Thus, we will explore in two longitudinal analyses: between $PM_{2.5}$ and O_3 exposure and CIMT and plaque, two well-established markers of subclinical atherosclerosis, utilizing the Study of Women's Health Across the Nation (SWAN) cohort.

1.7 SUMMARY TABLE

Table 1-2 Current Studies on Air Pollution and Carotid Intima-Media Thickness and Plaque Burden

Author, year	Population	Follow-up time†	Exposure	Unit	Outcome	Site	Main findings
Prospective Studies							
Kauffman, 2016	N=3,547 (CIMT); N=5,834 (CAC)	10-years	$PM_{2.5}$ NOx NO_2 Black carbon	5 μg/m^3 40 ppb 10 ppb 0.5μg/m^3	CAC, CIMT	Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; Northern Manhattan and Southern Bronx, NY; and St Paul, MN	Pollutants were not associated with CIMT, the point estimates were very close to zero. Each 5 μg/m^3 increase of $PM_{2.5}$ was associated with 4.1 Agatston units per year (95% CI: 1·4 – 6·8) progression of CAC; a 40 ppb increase of NOx was related to 4·8 Agatston units per year (0·9–8·7) progression of CAC; per 10 ppb increase of NO_2 contributed to 2·7 Agatston units per year (–0·3 to 5·7). BC was not related to CAC.
Gan, 2014	N=509 30-65 years old From M-CHAT*	5 years	≤150 m form a high way, or ≤ 50 m from a major road; Black carbon, $PM_{2.5}$, NO_2, NOx and noise	 10^{-5}/m μg/m^3 μg/m^3 μg/m^3 dB(A)	CIMT, plaque number, plaque area and the progression	Vancouver, Canada	No significant association was found between traffic proximity and any of the outcomes. No significant association was found between the air pollutants and progression of the outcomes. Chinese who lived near roads had an increased plaque area (1.12 mm^2, CI: 0.21-0.23), and European who lived near roads had a decreased plaque area (1.86 mm^2, CI: 0.65-3.06).
Adar, 2013	N=5,660 45-84 years old From MESA*	2.5 years	$PM_{2.5}$ (average annual $PM_{2.5}$, and baseline $PM_{2.5}$)	μg/m^3	CIMT and CIMT progression	Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; Northern Manhattan and Southern Bronx,	Each 2.5 μg/m^3 increase in baseline $PM_{2.5}$ have a 6.3 μm (CI: 2.8 – 9.8) higher in CIMT at baseline in the total population, and 3.8 μm (CI: 1.2 – 6.4) higher in CIMT progression overtime within site Within the site, per 2.5 μg/m^3 increase in mean follow-up $PM_{2.5}$, the progression of CIMT was 5.0 μm (CI: 2.6 – 7.4)

Table 1-2 Continued

Author, year	Population	Follow-up time[†]	Exposure	Unit	Outcome	Site	Main findings
						NY; and St Paul, MN	higher; and per 1 μg/m^3 change in $PM_{2.5}$, CIMT progression was 2.8 μm (CI: 1.6 – 3.9) higher
Wilker, 2013	N=380 61-96 years old Normative Aging Study: Men only	1.5 – 3 years	Black carbon	μg/m^3	CIMT and CIMT progression	Greater Boston Area, MA	Annual average black carbon during the year before baseline was associated with higher CIMT (1.1%, CI: 0.4 – 1.7) No association between black carbon and CIMT progression was found
Kunzli, 2010	N=1,483 Mean age=59 years old (40-89) Five RCTs: BVAIT (475) VEAPS (350) TART (169) EPAT (275) WELL-HART (214)	 3.3 years 2.8 years 1.8 years 1.8 years 2.9 years	$PM_{2.5}$ Proximity to highway (within 100 m) and main roads (within 50m)	μg/m^3	CIMT progression	Los Angeles Area	In the total sample, the people lived within 100 m of traffic got a 5.5 μm increased of CIMT progression per year (CI: 0.1-10.8, p=.04). $PM_{2.5}$ was only associated with CIMT progression in the treatment group (4.4 μm, CI: 0.4-8.4, p=.03) There were an interaction between low income and lower education and live closed to the roads on CIMT progression (p<.05).
Long-term exposure Studies							
Armijos, 2015	N= 287 Children aged 6-14 years old	Life time	Proximity to traffic: <100 m, 100-199 m and ≥200 m	--	CIMT	Quito Metropolitan District, Ecuador	Children lived within 100 meters to road was significantly more likely to have an increased mean and maximum CIMT than children lived more than 200 meters away from the road
Tsao, 2014	N=107 (Forest) N=114 (Urban) Mean age: 44 years	Occupational time	Forest vs. Urban Environment ($PM_{2.5}$, NO_x, CO, and SO_2 are lower in forest environment)	--	CAVI, ABI and CIMT	Xitou, Taiwan	No association was found between forest environment and CAVI ABI was lower among the forest staffs Mean internal IMT and IMT mean were lower among the forest staffs

Table 1-2 Continued

Author, year	Population	Follow-up time†	Exposure	Unit	Outcome	Site	Main findings
Rivera, 2013	N=2,780 58 ± 18 years (median ± IQR) from REGICOR	10 years	NO_2; traffic load in a 100 m buffer; and traffic intensity in nearest street	$\mu g/m^3$ veh-m/day veh/day	CIMT ABI	Girona region, Spain	NO_2, traffic in the nearest street, and traffic in the 100 m buffer were strongly associated with CIMT in the unadjusted models, but not in the further adjusted models. Traffic intensity in the nearest street (15,000 veh/day) was related to CIMT (2.32 μm, CI: 0.48 – 4.17) There was no association between the exposures and ABI.
Brenton, 2012	N=768 College student 20 (1.5) years old	0-5 year-old/ 6-12 year-old/ life time	O_3 NO_2 $PM_{2.5}$ PM_{10}	ppb ppb $\mu g/m^3$ $\mu g/m^3$	CIMT	Mainly in CA, but all over the US	9.3 ppb exposure to O_3 was related to 10.1 μm (CI: 1.8 – 18.5) increase in CIMT at childhood exposure (6 -12 years); and early childhood (0 – 5 years) and childhood exposure to O_3 was related to CIMT increase after adjusting for other pollutants.
Cross-Sectional Studies							
Perez, 2015	N= 9,183 Mean age around 60 years old Four cohort studies: IMPROVE KORA HNR REGICOR	One-year mean exposure at year 2008 or 2009	PM_{coarse}, $PM_{2.5\ abs}$, PM_{10}, NO_2 and NO_x ≤ 100 m from a major road of traffic intensity and traffic load	$\mu g/m^3$ $\mu g/m^3$ $\mu g/m^3$ veh*day^{-1}*10^{-4} veh*day^{-1}*m^{-1} *10^{-4}	CIMT	Stockholm, Sweden; Augsburg, Germany; Ruhr Area, Germany; Girona region, Spain	No significant association was found among the total population. Among IMPROVE-Stockholm, 10 $\mu g/m^3$ increased exposure of PM_{10} was associated with 5.9% decrease in CIMT, 10 $\mu g/m^3$ increased exposure to NO_2 was related to 5.0% decrease in CIMT, and 20 $\mu g/m^3$ increased exposure to NOx was related to 7.1% decrease in CIMT; and each 4,000,000 veh*day^{-1}*m^{-1}*10^{-4} increase to traffic load was associated with 5.5% CIMT increase.
Su, 2015	N=689 35-65 years old	One-year before CIMT measure	PM_{10}, $PM_{2.5}$, $PM_{2.5\ abs}$, and Nitric oxide (NO_2 and NO_x)	$\mu g/m^3$ 10^{-5}/m $\mu g/m^3$ $\mu g/m^3$	CIMT	Taipei, Taiwan	PM_{10}, $PM_{2.5\ abs}$, NO_2 and NO_x were associated with left side maximum CIMT (4.23 μm, CI: 0.32-8.13 per 10^{-5}/m $PM_{2.5abs}$; 3.72 μm, CI: 0.32-7.11 per 10 $\mu g/m^3$ PM_{10}, 2.81 μm, CI: 0.32 – 5.31

Table 1-2 Continued

Author, year	Population	Follow-up time†	Exposure	Unit	Outcome	Site	Main findings
							per 20 μg/m^3 NO_2, 0.74 μm, CI: 0.08 - 1.41 per 10 μg/m^3 NO_x) and only NO_2 was significantly related to combined CIMT; $PM_{2.5\ abs}$ was associated with left side mean CIMT (4.19 μm, CI: 0.38-8.00 per 10^{-5}/m $PM_{2.5abs}$), and per 10 μg/m^3 increase of PM_{10} was associated with 3.58 μm (CI: 0.27-6.89) left side and 2.68 μm (CI: 0.17-5.53) combined mean CIMT increase
Kim, 2014	N=5,488 45-84 years old From MESA	One-year before CIMT measure	$PM_{2.5}$ and chemical components of $PM_{2.5}$: sulfur, silicon, elemental carbon, and organic carbon	μg/m^3 μg/m^3 μg/m^3 μg/m^3 μg/m^3	CIMT	Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; Northern Manhattan and Southern Bronx, NY; and St Paul, MN	In the spatiotemporal model $PM_{2.5}$, sulfur, silicon and OC, were associated with CIMT, but not EC. In the national spatial model, sulfur, silicon and OC, were associated with CIMT. (The results were only presented in the figure, so an accurate point estimator is not available.)
Sun, 2013	N= 6,256 45-84 years old From MESA	One-year before CIMT measure	$PM_{2.5}$ and chemical components of $PM_{2.5}$: sulfur, silicon, elemental carbon, and organic carbon	μg/m^3 μg/m^3 μg/m^3 μg/m^3 μg/m^3	CIMT and CAC	Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; Northern Manhattan and Southern Bronx, NY; and St Paul, MN	$PM_{2.5}$ associated with CIMT, EC and OC were also significantly associated, especially OC got a bigger effect size (about 15.8μm, CI: 9.9-21.7, 10.8 μm, CI: 4.6-16.9 and 36.5 μm, CI: 28.0-44.9 per IQR of PM2.5, EC, OC and sulfur, respectively). But, there was not association with silicon (7.3, CI: -8.0-22.5). No significant association with CAC
Tonne, 2012	N=2,348 Civil servant in	52 week before the scan	PM_{10} and PM_{10}*OP	μg/m^3 m^{-3}	CIMT	Greater London, UK	5.2 μg/m^3 increase in PM_{10} is associated with 5% increase in CIMT (CI: 1.9-

Table 1-2 Continued

Author, year	Population	Follow-up time†	Exposure	Unit	Outcome	Site	Main findings
	Whitehall II study						8.3%) 1.3 m-3 increase in PM_{10}*OP is associated with 1.2% increase in CIMT (CI: 0.2-2.2%)
Lenters, 2010	N=750 (N=524 for PWV) 28.4 (0.9) years old Atherosclerosis Risk in Young Adults study	Annual mean at year 2000	NO_2 SO_2 $PM_{2.5}$ Black smoke Proximity to roads	μg/m^3 μg/m^3 μg/m^3 μg/m^3 Veh/day	CIMT PWV	Utrecht, Netherland	No significant association between any of the air pollutants and CIMT NO_2 and SO_2 are associated with 4.1% and 5.3% increase in PWV, and NO_2 is associated with 37% increase in augmentation index.
Bauer, 2010	N=3,380 HNR cohort	1-year air pollutant exposure at year 2001 or 2002	$PM_{2.5}$, PM_{10} and traffic intensity	μg/m^3	CIMT	Essen, Mülheim, and Bochum, German	Per 4.2 μg/m^3 increase in $PM_{2.5}$ is associated with 4.3% increase in CIMT, but the association between PM_{10} and proximity to traffic intensity and CIMT are not significantly associated
Künzli, 2005	N=798 Two RCTs: VEAPS BVAIT (1998-2003)	Annual mean at year 2000	$PM_{2.5}$	μg/m^3	Mean CIMT	Los Angeles Area, CA	10 μg/m^3 increase in $PM_{2.5}$ is associated with 5.9 μm increase in CIMT in total sample in the unadjusted model, but not adjusted models. This association remained significant among the women 60 years or older, and people who took lipids lowering drug in the adjusted models.

Abbreviations: CIMT: carotid intima-media thickness; ABI: ankle–brachial index; PWV: pulse wave velocity, CAC: coronary artery calcium; PM: particular matters; OP: oxidative potential; EC: elementary carbon; OC: organic carbon

BVAIT: B-Vitamin Atherosclerosis Intervention Tria; CAVI: cardio-ankle vascular index; EPAT: Estrogen in the Prevention of Atherosclerosis Trial; HNR: Heinz Nixdorf Recall; KORA: kooperative gesundheitsforschung in der region augsburg; M-CHAT: Multicultural Community Health Assessment Trial; MESA: Multi-Ethnic Study of Atherosclerosis; REGICOR: Registre Gironí del Cor: the Gerona Heart Register; TART: Troglitazone Atherosclerosis Regression Trial; VEAPS: Vitamin E Atherosclerosis Progression Study; WELL-HART: Women's Estrogen-Progestin Lipid-Lowering Hormone Atherosclerosis Regression Trial

Note: † Follow-up time: follow-up during two or more CIMT measures, or exposure period estimation to CIMT me asures in the long-term exposure study

2.0 SPECIFIC AIMS

This dissertation consists of three manuscripts with the following specific aims:

Specific Aim for Manuscript 1:

To estimate the association between acute exposure to $PM_{2.5}$ and criteria gaseous pollutants (O_3, NO_2 and SO_2) and CVD emergency department (ED) visits in Allegheny County, PA from 1999 – 2011, using a case-crossover design.

Specific Aim for Manuscript 2:

To examine the relationship between prior $PM_{2.5}$ and O_3 exposure and annual progression of subclinical atherosclerosis (CIMT and plaque) during a 2-year-period, using SWAN Heart data; adjusting for age, race, education, how hard to pay for basis and CVD risk factors, body mass index (BMI), smoking, triglyceride, LDL, HDL, menopause status, blood glucose, diabetes, and family history of cardiovascular disease or stroke.

Specific Aim for Manuscript 3:

To investigate the relationship of early exposure to $PM_{2.5}$ and O_3, measured during SWAN visits 3 – 7 to the subclinical atherosclerosis markers (CIMT and plaque) measured at SWAN visits 12 and 13, adjusting for traditional CVD risk factors.

3.0 A CASE-CROSSOVER ANALYSIS OF SPATIOTEMPORAL EXPOSURE TO AIR POLLUTANTS AND CARDIOVASCULAR EMERGENCY ROOM VISITS IN ALLEGHENY COUNTY, PENNSYLVANIA IN 1999 – 2011

3.1 ABSTRACT

Introduction: The acute effects of air pollution and cardiovascular disease (CVD) have been studied, but very few studies have focused on spatiotemporal modeled exposure to air pollutants at the population level. This study aims to examine the short-term association between four spatio-temporal modeled air pollutants ($PM_{2.5}$, O_3, NO_2 and SO_2) and CVD emergency room visits in Allegheny County from 1999 – 2011 using a case-crossover study design.

Methods: Land-use regression was used to model the ground level exposures to $PM_{2.5}$, O_3, NO_2 and SO_2. We included the parameters of monitored data, land cover variables, meteorological data, and aerosol optimal depth for $PM_{2.5}$ modelling, in addition, ZIP code level exposure to these pollutants were estimated. CVD emergency room visits were requested from the local hospitals of the two health networks in Allegheny County, which operate the majority of the emergency room services. The discharge ICD-9 code was used to identify the CVD cases and CVD subgroups. This dataset also provided the information on date of emergency room visit, age, gender, race, disposition, and ZIP code of residence. A case crossover design was used in which control days were matched on the same day of week +/- 1 and 2 weeks that a visit (i.e.,

case) occurred. We linked the ZIP code level air pollution data with the patients' ZIP code of residence to determine the individual level exposure estimation of both case days and control days. Conditional logistic regression with multi-pollutant and distributed lags of 0 – 3 days was applied to estimate the effect of acute exposure of these pollutants to CVD emergency room visits, adjusting for temperature.

Results: There were 181,789 cases identified during the 13-year period. In the overall analyses, we found that per IQR increase of O_3 exposure (25.52 ppb), there was 6.6% (95% CI: 0.8% - 12.7%) increase in the odds of an AMI emergency room visit. We also observed that with each IQR (8.4 $\mu g/m^3$) increase of $PM_{2.5}$ at lag 1 and each concurrent IQR (7.5 ppb) increase of NO_2 was associated with a 10.7% (95% CI: 1.6% – 20.5%) and 7.6% (95% CI: 0.3% – 15.5%) increased odds of peripheral vascular disease emergency room visits, respectively. Among women and Black, we observed an association of $PM_{2.5}$ with acute myocardial infarction, and with ischemic heart disease. We also found that temperature had an effect on CVD emergency room visits, both as a warm season and a cold season effect.

Conclusion: We found an association of $PM_{2.5}$ and NO_2 with CVD emergency room visits, and this association persisted in the stratified analyses, as well as in the later years with lower exposure levels. The findings may suggest that further actions to reduce the pollution level in this area should be taken.

Key words: Multi-pollutants, Cardiovascular Emergency Room Visits, Spatio-temporal, Acute, Case-crossover

3.2 INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death both in the U.S. and worldwide (Centers for Disease Control and Prevention 2015, World Health Organization 2015). Short-term health effects of air pollution on CVD have been studied over the years. (Brook, Rajagopalan et al. 2010, Franchini and Mannucci 2012, Atkinson, Carey et al. 2013). Several plausible biological mechanisms connecting air pollution and CVD events have been proposed (Brook, Rajagopalan et al. 2010, Franklin, Brook et al. 2015). Pollutants can increase the risk of arrhythmia, increase systemic inflammation and oxidative stress, and increase the plaque vulnerability, and thus, trigger CVD events. Based on this evidence, the U.S. Environmental Protection Agency has implemented dozens of air quality related policies and regulations over the past few decades to reduce air pollution levels in the U.S. (Dominici, Peng et al. 2007, U.S. Environmental Protection Agency 2016, U.S. Environmental Protection Agency 2016). Thus, the level of $PM_{2.5}$, NO_2 and SO_2 has decreased dramatically (U.S. Environmental Protection Agency 2016). We are seeking to answer: are these lower levels of air pollutants associated with a lower risk of CVD emergency events over time. Shi and colleagues focused on the low concentration of $PM_{2.5}$ exposure less than 10 $\mu g/m^3$ and CVD mortality; however an effect of $PM_{2.5}$ on lag0 and lag1 for mortality was still observed. (Shi, Zanobetti et al. 2016).

In a real-world setting, individuals are not exposed to one pollutant at a time, as multiple forms of air pollution co-exist in the environment. Most of the current literature is focused on one pollutant, and some only consider a single lag or moving average, instead of the use of multi-lags, or using the exposure matrixes as moving average of 2 – 3 days exposure (Ballester, Rodriguez et al. 2006, Dominici, Peng et al. 2006). For the papers with multi-pollutants tested, authors often are only able to access and investigate two-pollutant models, i.e. the effect of one

pollutant adjusted for the other (Ballester, Rodriguez et al. 2006, Talbott, Rager et al. 2014). In this paper, we are interested on the effect of multiple pollutants on CVD emergency room visits. Whether the effect of one pollutant still exists after adjusting for other pollutants is another question we want to answer in this paper.

In recent years, there has been an increasing number of studies using modeled data for exposure assessment in epidemiologic studies (Kloog, Nordio et al. 2014, Shi, Zanobetti et al. 2016). Most of this literature utilizes aerosol optical depth (AOD) as the modeling method to address the spatiotemporal exposure of $PM_{2.5}$ among the population, since AOD can only model the exposure level of particles (Kloog, Nordio et al. 2014). While taking into account gaseous pollutants, surface level spatio-temporal modeling is required (Rodopoulou, Chalbot et al. 2014, Shmool, Kinnee et al. 2016). A recently published paper using spatiotemporal modeling of both $PM_{2.5}$ and ozone exposure revealed a higher variation of the exposure among the study population, and a stronger association between O_3 and asthma hospitalization and emergency room visits (Shmool, Kinnee et al. 2016). Furthermore, spatiotemporal modeling can be easily adopted in the case-crossover design.

We obtained information on CVD emergency room visits from local hospitals. There were very few studies using the emergency room visits data for such analyses, as this information may involve a direct request from individual hospitals (Metzger, Tolbert et al. 2004, Rodopoulou, Samoli et al. 2015). Current literature generally focused on the CVD mortality or hospitalizations (Dominici, Peng et al. 2006, Peng, Bell et al. 2009, Kim, Peel et al. 2012, Powell, Krall et al. 2015). Mortality analysis implies use of the date of death not the date of the event and may therefore result in a longer lag (Dabass, Talbott et al. 2016) compared to the CVD hospitalization investigations , which are associated with effects concurrently (lag 0) or 1 day

after (lag 1) (Haley, Talbot et al. 2009, Linares and Diaz 2010, Ito, Mathes et al. 2011, Rodopoulou, Samoli et al. 2015). Among the studies using the hospitalization data, some eliminated the elective events, such as scheduled office visits or out-patient surgery (Peng, Bell et al. 2009). But, these cannot capture all the acute cases. From our current data, there were about 26% of CVD emergency room visits that were discharged on the same day.

Recent literature has focused more on $PM_{2.5}$ and O_3 (Winquist, Klein et al. 2012, Rodopoulou, Chalbot et al. 2014, Talbott, Rager et al. 2014, Pope, Muhlestein et al. 2015, Dabass, Talbott et al. 2016), while, very few have studied an additional two gaseous criteria pollutants: NO_2 and SO_2. These two pollutants were also related to cardiovascular outcomes in population based studies (Metzger, Tolbert et al. 2004, Pereira Filho, Pereira et al. 2008, Linares and Diaz 2010, Ito, Mathes et al. 2011), and possibly through the oxidative stress pathway. In this paper, we examined the acute association between four spatio-temporal modeled criteria air pollutants ($PM_{2.5}$, O_3, NO_2 and SO_2) and CVD emergency room visits in Allegheny county from 1999 - 2011 using a case-crossover study design.

3.3 METHODS

3.3.1 Study domain

The study domain was Allegheny County (Allegheny County includes the city of Pittsburgh), Pennsylvania (PA), which is in southwestern of PA. It is at downwind from sources of pollution in the Midwest. Thus, the Pittsburgh region still ranks as one of the more polluted areas in the U.S. (National Aeronautic and Space Administration 2010).

3.3.2 Emergency Room Visits Data Collection

We requested emergency room visits data including information on both emergency room discharge and emergency room admissions between January 1,1999 to December 31, 2011, inclusive, from the hospitals of the two local healthcare networks, University of Pittsburgh Medical Center (UPMC) and Allegheny Health Network. These two healthcare networks own the majority of the hospitals and emergency departments in Allegheny County. Primary discharge diagnoses based on the first 3 digit of ICD-9 code of 390 - 459 were used to identify all the CVD cases. The requested dataset contained the following variables: hospital, pseudo ID, admit date, discharge date, age, sex, race, ZIP code of residence, county of residence, primary and secondary discharge diagnosis (ICD-9 code), discharge disposition (discharged or admitted to in-patient). The study population was restricted to the population 40 years old and older, and county of residence was restricted to Allegheny County only. We excluded the cases of the same patient readmitted to emergency room within 30 days by the same diagnosis (Dunlay, Weston et al. 2012), because these readmitted cases may be more likely to be due to the continuation of symptoms related to the earlier event. Besides all CVD events, we also look at the CVD subgroups. These subgroups are heart failure (HF, ICD-9: 428), ischemic heart disease (IHD, ICD-9:410 - 414 and 429), acute myocardial infarction (AMI, ICD-9: 410), arrhythmia (AR, ICD-9: 426 - 427), stroke (ICD-9: 430 - 438), peripheral vascular disease (PVD, ICD-9: 440-448) (Dominici, Peng et al. 2006).

3.3.3 Exposure Modeling

The monitor data of four air pollutants ($PM_{2.5}$, O_3, NO_2, and SO_2) were obtained from multiple sources to cover the study domain and 32-county area beyond the study area. The monitors are from the sources: the Air Quality System (AQS) from the U.S. EPA, the Pittsburgh Air Quality System (PAQS), the Steubenville Comprehensive Air Monitoring Program (SCAMP), and the Clean Air Status and Trends Network (CASTNET) (Dabass, Talbott et al. 2016). The sites of the monitors are shown in Figure 3-1. All the land use information and meteorological data were scaled into 1 km X 1 km grid before putting them into the model. Land covers were collected from National Land Cover Database, 2006 (Fry, Xian et al. 2011). Elevation were obtained from the National Elevation Database (U.S. Geological Survey 2017). The roads information was obtained from the Topologically Integrated Geographic Encoding and Referencing (TIGER) products from the U.S. Census Bureau (U.S. Census Bureau 2012). The traffic intensity in each grid was determined by the length of primary roads and total counts of the intersections within the 1 km X 1 km grid. Meteorological variables were obtained from North American Regional Reanalysis (NARR) products generated by National Centers for Environmental Prediction (NCRP) from their website (NOAA 2016). The level-2 aerosol product of combined land and ocean with a spatial resolution of a 10 X 10 km resolution array was achieved from the Moderate Resolution Imaging Spectroradiometer Atmosphere Group (Atmosphere 2014). This aerosol optical depth (AOD) variable was only used for $PM_{2.5}$ spatial modeling. A two-step method was applied to associated air pollution level of each pollutants and these covariates. The detailed methods of the modelling have presented elsewhere (Xue 2015, Bilonick, Talbott et al. 2016, Talbott, Bilonick et al. 2016). This two-step approach was applied in the spatio-temporal

estimates of each pollutant separately. A selected day exposure estimation to demonstrate spatial variance of these four pollutants from this modelling was shown in Figure 3-2.

The temporal mean temperature is monitored at the Pittsburgh Air site cover the whole study period, and the data was retrieved from the National Oceanic and Atmospheric Administration (NOAA 2014).

3.3.4 Statistical Models

The referent days were selected as the same day of the week and +/- two weeks of the CVD case admitted day. Thus, there are four referent days per case. Conditional logistic regression with distributed lags of lag 0 - lag 3 was applied to estimate per IQR increase of the air pollutant associated with the odds ratio of the CVD emergency room visits for all CVD and CVD subgroups (Haley, Talbot et al. 2009). Because previous studies showed that short lags were related to CVD outcomes, we chose not to include additional lags in the model (Dominici, Peng et al. 2006, Peng, Bell et al. 2009, Kim, Peel et al. 2012, Powell, Krall et al. 2015). The main analyses were based on using a multi-pollutant model which included all four air pollutants and adjusted for the mean temperature. The single pollutant model with distributed lags controlling for the mean temperature was presented in the supplementary materials. We also stratified the analyses by age (10-year age groups), sex, race, disposition, season (spring: March - May, summer: June - August, fall: September - November, and winter: December - February) and early (1999 - 2005) and late (2006 - 2011) of study period. SAS software version 9.3 was used for the statistical analyses (SAS Institute Inc., Cary, NC, USA).

3.4 RESULTS

Over the course of 13 years, there were a total of 181,789 ED visits within Allegheny county that were identified. There were slightly more women (54.7%) admitted to the ED, which is compatible with the population composition according to 2010 American Community Survey of the population over 40 years old (U.S. Census Bureau 2016). But, men were more likely go to emergency room due to IHD. The number of cases included 18.1% who were African American, which is higher compared to the whole population of 12.9% of African American in Allegheny County. This higher percentage was driven by stroke and PVD. The cases had a much lower proportion of other racial/ethnicity groups, 0.8% vs. 6.7% in the population. There were about 57.3% of these cases that were over age 70 years old and more than 30% of cases were over age 80 years old. There are about 26.4% of cases which were discharged on the same day, and more of these cases had a discharge diagnosis of AR and PVD. The detailed numbers and percentages were showed in Table 3-1.

Table 3-2 summarizes the spatiotemporal exposure level of these pollutants by cases on the same day. The mean $PM_{2.5}$ exposure was about 14.2 $\mu g/m^3$, with the maximum value at 57.44 $\mu g/m^3$; the mean O_3 exposure was about 37.9 ppb, with the highest value at 137.8 ppb. The maximum values of NO_2 and SO_2 over the study period were relatively low. The correlations between the pollutants were low to moderate (Table 3-3). This indicated that multi-collinearity among the pollutants would not be a problem. Over the time period of the study, there was a long-term trend of pollutant level decrease for $PM_{2.5}$ at 0.35 $\mu g/m^3$ per year, NO_2 at 0.36 ppb per year, and SO_2 at 0.46 ppb per year; but, there was no significant change of O_3 exposure level over this study period (data not shown).

In the primary analyses with the whole study population, we found that O_3 on lag Day 2 was associated with the AMI emergency room visits. Per IQR increase of O_3 exposure (25.52 ppb), there was 6.6% (95% CI: 0.8% - 12.7%) increase in the odds of an AMI emergency room visit. We also found that an IQR increase of $PM_{2.5}$ on lag Day 1 was associated with a 10.7% (95% CI: 1.6% - 20.5%) increase in the odds of a PVD emergency room visit; and, an IQR increase of NO_2 on lag 0 was related to 7.6% (95% CI: 0.3% - 15.5%) increase in odds of PVD emergency room visit.

After stratification by gender, we noted that air pollutants were related to these two outcomes, but with different pollutants at different lags. Among men, with each IQR increase of SO_2 at lag 2, there was a 6.2% (95% CI: 1.1% - 11.4%) increase in AMI emergency room visit; and with each IQR increase of O_3 at lag 2, there was a 26.7% (95% CI: 7.8% - 48.8%) increase in PVD emergency room visit. While among women, we found that $PM_{2.5}$ at lag 1 was associated with IHD, AMI and PVD emergency room visits (Figure 3-5). The racial groups also had different responses to the air pollutants exposures. We observed that with each IQR increase of NO_2 at lag 3, the white cases were 3.1% (95% CI: 0.1% - 6.1%) more likely to go to the emergency room for HF; with each IQR increase of O_3 at lag 3, the white ED cases were also 5.7% (95% CI: 0.2% - 11.6%) more likely to be seen in the emergency room for AMI, and this outcome was also associated with NO_2 at lag 3, with 4.3% (95% CI: 0.2% - 8.5%) increase of odds. On the other hand, we found that $PM_{2.5}$ at lag 1 and lag 2 were related to AMI, as well as SO_2 at lag 2 among the Black patients of the ED visits. After stratification by age, we noted that among the younger age groups, stroke patients were more likely to be impacted by air pollutants. In the 40 - 49 years old age group, each IQR increase of NO_2 at lag 0 was related to 14.2% (95% CI: 1.9% - 28.0%) increase in stroke emergency room visit; and in the 50 - 59 years old age

group, each IQR increase of SO_2 at lag 1 was associated with 9.7% (0.7% - 19.6%) increase in stroke emergency room visit. While in the 70 - 79 years old age group, we found that with each IQR increase of $PM_{2.5}$ at lag 3, people were 5.7% (95% CI: 0.6% - 11.0%) more likely went to emergency room due to stroke. Among our youngest age group, we also found that $PM_{2.5}$ at lag 0 is related to a 15.8% (95% CI: 1.5% - 32.0%) increase in emergency room visits with a diagnosis of HF; and among those who were 80 years and older, we also observed that each IQR increase of NO_2 at lag 3 were related to 3.7% (95% CI: 0.6% - 6.9%) increase of HF emergency room visits. All the other findings with age stratification were related to IHD, AMI and PVD (Figure 3-8 to Figure 3-12).

As previously stated, the data collected directly from the emergency room visits can also capture the patient discharged on the same day. The proportion in this category got a slightly higher proportion of those who were African American (22%). Among these patients, we found that with an IQR increase of SO_2 on the previous day, there was a 7.9% increase (95% CI: 2.1% - 14.1%) in emergency room visits due to stroke; and with an IQR increase of $PM_{2.5}$ on the previous day, there was a 21.6% increase (95% CI: 4.0% - 42.2%) in the PVD emergency room visits. The confidence intervals for these two observations were large however. While among the patient transfer to in-patient, we only observed the each IQR increase of O_3 on lag 2 was related to AMI emergency room visits (OR: 1.07, 95% CI: 1.01 - 1.14).

When stratified by season, we also presented the temperature effect in each model (Figure 3-15 to Figure 3-18). As this study was using a case-crossover design, we cannot add in season as spline in the overall model, and temperature is varied largely by season. So, we presented the effect of mean temperature only in this stratification analyses. In the spring, we found that at lag 0, per IQR higher mean temperature was related to All CVD (OR=1.12, 95%

CI: 1.06 - 1.19), IHD (OR=1.15, 95% CI: 1.00 - 1.32) and AMI (OR=1.23, 95% CI: 1.02 - 1.49). In the fall, we observed a similar association between mean temperature and these outcomes with a slightly higher effect size; in addition, we also found that the same day IQR increase of temperature also contribute to 24.6% (95% CI: 6.4% - 46.0%) higher stroke emergency room visit. In the summer, however, we only observed that same day temperature was related to AR (OR=1.29, 95% CI: 1.04 - 1.62), and the temperature on lag 3 was related to AMI (OR=1.41, 95% CI: 1.05 - 1.89). On the other hand, in the winter, each IQR lower temperature on the same day contributed to a 27.9% (95% CI: 3.1% - 46.8%) increase of PVD emergency room visits; and lower temperature on the lag 3 day contributed to a 12.6% (95% CI: 0.9% - 32.9%) increase of AMI emergency room visit. We also noted the effect of NO2, SO2 on some outcomes in the warm period of the year, but the effect of $PM_{2.5}$ was revealed in the summer and winter. The O_3 was not significantly related to any of the outcomes in the summer, but in other seasons.

During the early period of years 1999 - 2005, the same day exposure NO_2 and O_3 was significantly associated with All CVD, IHD and AMI; while in the late period of years 2006 - 2011, the effect NO_2 and O_3 on IHD and AMI was also noted, but at a longer lag, lag 2 or lag 3 (Figure 3-19 and Figure 3-20). We additionally found that $PM_{2.5}$ at lag 1 (OR = 1.21, 95% CI: 1.06 - 1.39) and SO_2 at lag 0 (OR = 1.18, 95% CI: 1.06 - 1.32) was related to PVD at the later period. However, the magnitude of the point estimates between the exposure and outcomes was not very much different.

3.5 DISCUSSION

In this paper, we assessed the short-term effect of multiple pollutants ($PM_{2.5}$, O_3, NO_2, and SO_2) on emergency cardiovascular disease in Allegheny County, PA over the years 1999 – 2011. We noted that overall, the addition of all four criteria air pollutants provided a fuller picture of their effects on various population sub groups. This may reflect differences in their exposure and or specific vulnerabilities. In the whole population, we found that O_3 was related to AMI at lag 2, and $PM_{2.5}$ at lag 1 and NO_2 at lag 0 related to PVD. While in the stratified analyses, we also noted the effect of these pollutants and CVD outcomes. These associations were majorly revealed with IHD, AMI and PVD, and in some strata, the relationship with stroke and HF was also shown.

In the overall analyses, we found that an effect of O_3 on AMI. The effect of O_3 and CVD had been established in several studies (Bell, Dominici et al. 2005, Goodman, Prueitt et al. 2014). In a meta-analyses, Bell and colleagues showed that each 10-ppb increase of O_3 exposure contributed to 1.11% (95% CI: 0.68% – 1.53%) increase of CVD mortality. On the contrary, Goodman et al use weight-of-evidence in a review addressed the association between O_3 and CVD morbidity or mortality and did not conclude that there were strong evidences supporting it (Goodman, Prueitt et al. 2014). The findings from the more recent literature on O_3 and AMI morbidity are varied. In a fourteen-city study in Spain, each 10-ppb increase of O_3 exposure at lags 2 – 3 was related to 0.7% (95% CI: 0.3% – 1.0%) higher CVD hospitalization (Ballester, Rodriguez et al. 2006). A study based on AMI registry in southwest of France also established an association between O_3 at lag 0 and lag 1 and AMI mortality and morbidity (Ruidavets, Cournot et al. 2005). Another recently published study conducted in Tuscany, Italy found that a 10-ppb increase of O_3 exposure of lags 1 – 5 was related to 6.3% (95% CI: 1.2% – 11.7%) increase of

out-of-hospital acute coronary events mortality, but not with hospital admissions (Nuvolone, Balzi et al. 2013). However, another study in Perth, Australia did not find a significant association between O_3 and CVD hospital admission (Hinwood, De Klerk et al. 2006). Wang et al did not observed an effect of O_3 on AMI hospital admission in Alberta, Canada, where have much lower O_3 level compared to the U.S. (Wang, Kindzierski et al. 2015). O_3 is a secondary photochemical pollutant, which means the formation of O_3 relied on the precursors and ultraviolet strength (U.S. Environmental Protection Agency 2015). These varied findings in the current literature may be due to the different levels of ground level O_3 in each area. O_3, as well as NO_2 and SO_2, are oxidizing agents, so that they can add oxidative stress to the body system; and thus, can harm human health (Yang and Omaye 2009, U.S. Environmental Protection Agency 2016). Thus, there are plausible mechanisms to connect O_3 and CVD outcomes. In conclusion, this study shows a strong association between O_3 and increased risk of AMI emergency room visits. This effect still existed among whites, and in the early study period (1999 – 2005), and more profound in the late study period (2006 – 2011), and spring.

There are very few studies that examined the association between air pollutants and PVD. Dominici et al found that 10 $\mu g/m^3$ $PM_{2.5}$ at lag 0 was related to 2.11% (95% CI: 0.79% – 3.40%) higher PVD hospitalization from the Medicare claims of the national sample of the U.S. for 1999 – 2002. The study of the same area and same time period also found a larger effect size related to PVD mortality (Dabass, Talbott et al. 2016). Dabass et al found that an increase of 10 $\mu g/m^3$ $PM_{2.5}$ at lag 5 contributed to 7.6% (95% CI: 0.05% – 15.7%) PVD mortality. One the other hand, we noted that per IQR increase of $PM_{2.5}$ at lag 1 resulted in a 10.7% (95% CI: 1.6% – 20.5%) increase in odds of PVD emergency room visit. The sample size for PVD is much smaller than that seen for AMI, IHD or CVD as a whole making this finding somewhat more

problematic and demonstrating very large confidence limits. Although there were very few studies using PVD as an outcome, there were association between short-term $PM_{2.5}$ exposure and PVD emergency room visits. Unfortunately, there was no published literature trying to establish a relationship between NO_2 and PVD. As an oxidative agent, NO_2 can plausibly contribute to PVD events (Du, Xu et al. 2016).

When the population was stratified by gender, we still noted the association between air pollution and IHD, AMI and PVD. However, males and females may have differential indoor versus outdoor exposures. Male AMI patients were more susceptible to SO_2, and male PVD patients were more likely to be affected by O_3 exposure. Females responded to $PM_{2.5}$ with all these outcomes. As most of the current literatures were using $PM_{2.5}$ as an exposure, and not all the papers look at effect modification, the discussion for gender differences were restricted to the $PM_{2.5}$ exposure. Bell et al found that among females, the impact of $PM_{2.5}$ was larger on AR in the U.S. population, as well as in the northeast of the U.S. (Bell, Son et al. 2015). However, this gender difference is not consistent over the studies. Pope III and colleagues also found that the association between $PM_{2.5}$ and AMI were larger among the male than among the female in two other studies (Pope, Muhlestein et al. 2006, Pope, Muhlestein et al. 2015). On the other hand, Rodopoulou et al. only noted the association between $PM_{2.5}$ and CVD emergency room visits among the men in the cold period of the year, not among the women, in the central Kansas (Rodopoulou, Samoli et al. 2015). However, this data was collected at one hospital majorly serving uninsured, Medicaid and Medicare patients, who may be at a more disadvantage stage compared to the patient go to other health providers.

When examining the association between air pollution and CVD emergency room visits by race, we found that among white men and women, there was an association of O_3 at lag 2 and

increased risk of AMI, and additionally we noted that NO_2 at lag 3 was related to HF and AMI; while among African American men and women, $PM_{2.5}$ and SO_2 contributed to AMI. The different observations among the two racial groups may indicate disparities in residential characteristics, residential locations (living close to emission sources) and or a potentially different effect by race (Glad, Brink et al. 2012). In some the studies, they observe some racial difference, with different exposure or outcome (Rodopoulou, Samoli et al. 2015, Wing, Adar et al. 2015, Montresor-Lopez, Yanosky et al. 2016). However, there is not enough evidence for racial difference in the literature (Bell, Zanobetti et al. 2013).

In the seasonal analyses, we observed more associations with the air pollutants and temperature and CVD emergency room visits. In these analyses, we only note the effect of $PM_{2.5}$ on IHD in the summer; and on HF in the winter; and the gaseous pollutants were related to different CVD outcomes by season. Bell et al revealed the association between $PM_{2.5}$ and CVD hospital admissions was larger in the winter, and smaller and still statistically significant in other seasons in the Northeast of the U.S. (Bell, Ebisu et al. 2008). Another study in New York State also observed a larger effect of $PM_{2.5}$ on CVD mortality in winter, and smaller in the summer and fall (Hsu, Hwang et al. 2017). However, in the multi-cities analysis in the U.S., Dai et al found a stronger association between $PM_{2.5}$ and CVD mortality in the spring and summer than fall and winter (Dai, Zanobetti et al. 2014). In another study in Beijing, Su et al found an association between $PM_{2.5}$, SO_2 and NO_2 and CVD emergency room visits only in spring, but not in any other seasons (Su, Breitner et al. 2016). As previously stated, there was much seasonal variation of pollution level, components, as well as human behavior. However, in this study, we do not have these variables. Further investigation with $PM_{2.5}$ constituents will be conducted among this study population.

In the early and late periods of the study, we did not find a significant decrease in the association with of air pollution and health outcomes. This may in part be due to the fact that ozone did not decrease over time; whereas the other three pollutants had an almost 25% reduction in levels. In the early period, we found that the same day O_3 was related to IHD and AMI, and the same day NO_2 was associated to all CVD and IHD. While in the late period, we observed that the effects of pollutants occurred at a longer lag. O_3 at lag 2 was now related to AMI and the effect size was larger (10.1% vs. 6.6% at early period at lag 0); and NO_2 at lag 3 was not related to IHD and AMI, and the effect sizes were similar as the early period. We additional observed the effect of $PM_{2.5}$ at lag 1 and SO_2 at lag 0 on PVD in the late period. Dominici et al. considered this question for an earlier time period (for the years of 1987 – 2000) and noted weak evidence of an effect of PM on CVD mortality decline before and after 1995 (Dominici, Peng et al. 2007). Cox et al examined the study period after Dominici's study, of years 2000 – 2010, and again, they did not find a decline of CVD mortality associated with $PM_{2.5}$ or O_3 change over the study period, nor for 1 – 3 years intervals during this time (Cox and Popken 2015). We on the other hand were considering ED visits which may be very different than mortality as emergency treatment of heart related conditions over this 13 years period may have changed dramatically with new treatments and interventions. Our findings of the short-term exposures over the early versus later study period showed very little difference for $PM_{2.5}$ for most of the CVD outcomes (Figure 3-19 and Figure 3-20). Metzger et al even found a slightly larger effect of PM_{10}, NO_2, CO, and SO_2 at a later period (year 1998 – 2000) than the whole study period (1993 - 2000) in Atlanta (Metzger, Tolbert et al. 2004). However, we noted a longer lag at late period than the early period. We found the same-day effect of NO_2 and O_3 was significantly associated with All CVD, IHD and AMI; while in the late period years 2006 -2011,

the effect NO_2 and O_3 at lag 2 or lag 3 were observed. A study conducted in the northeast of the U.S. has found that the level of $PM_{2.5}$ in the rural areas is lower than 6 $\mu g/m^3$, and they proposed a potential threshold effect at this level (Shi, Zanobetti et al. 2016). This threshold is based on the data from the rural area, which may be biased due to less population and fewer events in the rural area. A systematic review and meta-analysis also found a potential threshold effect at 5.8 $\mu g/m^3$ in the relationship of air pollution and heart failure (Shah, Lee et al. 2015). This threshold is based on the potential linear dose-response relationship of the literatures, but there was no study conducted in the area with exposure level that low. So, this threshold was not observed. There may be a threshold for $PM_{2.5}$ and CVD outcomes which has yet to be observed or a linear no threshold dose may prevail.

There are some limitations of our study. The CVD emergency room visits data were requested from the hospitals, so all the cases from this case-crossover study were only restricted to those who ended up in the emergency room. So, our sample may be biased to the population who were more susceptible to air pollution, or at higher risk to CVD. This bias may be more profound in the younger age groups. Thus, we observed some unique connections in these age groups. Another potential limitation is that the we did not have any information on risk factors among these patients, like smoking, BMI, and comorbidities. So, we cannot examine how other modifiable factors in relationship of air pollution and CVD outcomes. Another limitation is that we are using the ambient pollution level, not accounting for the indoor exposure level, as we do not know whether they go out or not, or whether they spent more time at home or at work. Although we matched with weekday and hypothesized that their behavior was the same on the same weekday, we do not have any evidence to confirm it. Another limitation is the potential

multiple comparisons related to this paper. A test was considered statistically significant when the p-value < 0.05, and no adjustment was made to this significance threshold.

There are several strengths of our studies. We are using modeled data as our exposure assessment. This can show a better variation of the exposure of the participants (Shmool, Kinnee et al. 2016). Also, using the county average level of the exposure, may not reflect the true exposure level of the participants. Wing et al conducted a sensitivity analyses restricting the patients who lived 5 km near the monitors, and the effect size was attenuated (Wing, Adar et al. 2015). Unlike some of the recent literature using AOD and only have the estimation of $PM_{2.5}$ alone, we also estimated the ZIP code level exposure to gaseous pollutants. Moreover, our data collection was from multiple hospitals from the Allegheny County, PA, which capture almost all the cases of the areas. Other emergency studies, they may only restrict to one hospital (Barnett, Fraser et al. 2012, Liu, Breitner et al. 2013, Rodopoulou, Chalbot et al. 2014, Rodopoulou, Samoli et al. 2015). Using this data, we also can capture the patient who was discharged and not admitted to the hospital. The discharged patients were younger than the in-patient (mean age: 66.4 years vs. 73.3 years), and there was a slightly higher proportion of African American men and women. These patients were probably less ill and could go to home on the same day of admission. Still, we found the effect of $PM_{2.5}$ on PVD and SO_2 on stroke among them. Moreover, our study used a case-crossover design, where the cases served as their own control. Although we have very limited information from the discharged data, there were no confounders in this analyses, as all the other factors besides air pollution were matched and controlled (Talbott, Rager et al. 2014). Last but not the least, we used a multi-pollutant model with distributed lags, so we could examine the effect of each pollutant when adjusting for other pollutants, and the effects across all the selected lags.

We found an association between air pollution and CVD emergency room visits, and this association persists in the stratification analyses, and in the later years with lower exposure levels. The findings may suggest that further actions to take to reduce the pollution level in this area.

3.6 TABLES AND FIGURES

Table 3-1 Study Population Characteristics by Cardiovascular subgroups

	All CVD (n=181789)		Heart Failure (n=36045)		Acute Myocardial Infarction (n=18751)		Ischemic Heart Disease (n=34627)		Arrhythmia (n=30646)		Stroke (n=31128)		PVD (n=4669)	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Gender														
Female	99435	54.7	20457	56.8	9012	48.1	16463	47.5	16621	54.2	17854	57.4	2430	52.1
Male	82350	45.3	15588	43.2	9739	51.9	18162	52.5	14025	45.8	13273	42.6	2239	47.9
Race														
White	144110	81.1	29025	81.4	16233	87.5	29413	85.9	25860	87.0	25273	82.6	3687	81.5
Black	32103	18.1	6437	18.1	2160	11.6	4512	13.2	3645	12.3	5003	16.4	810	17.9
Other	1405	0.8	187	0.5	156	0.8	301	0.9	218	0.7	316	1.0	28	0.6
Deposition														
Discharge	47969	26.4	3902	10.8	2103	11.2	6026	17.4	10896	35.6	5698	18.3	1440	30.8
Transfer to In-patient	132738	73.0	32055	88.9	16634	88.7	28369	81.9	19555	63.8	25245	81.1	3214	68.8
Unknown	1082	0.6	88	0.2	14	0.1	232	0.7	195	0.6	185	0.6	15	0.3
Age (years)														
40-49	16521	9.1	1362	3.8	1373	7.3	2980	8.6	2464	8.0	1799	5.8	379	8.1
50-59	25663	14.1	2954	8.2	2868	15.3	5898	17.0	4263	13.9	3689	11.9	602	12.9
60-69	30003	16.5	5005	13.9	3258	17.4	6541	18.9	5284	17.2	5072	16.3	812	17.4
70-79	46088	25.4	9731	27.0	4650	24.8	8928	25.8	8183	26.7	8466	27.2	1259	27.0
80+	63514	31.9	16993	47.1	6602	35.2	10280	29.7	10452	34.1	12102	38.9	1617	34.6

CVD: cardiovascular disease, PVD: peripheral vascular disease

Table 3-2 Spatio-temporal air pollution estimates

	mean	Std	min	max	IQR
Cases (N=181789)					
$PM_{2.5}$ ($\mu g/m^3$)	14.17	7.20	2.77	57.44	8.41
O_3 (ppb)	37.91	17.67	-7.11	137.84	25.52
NO_2 (ppb)	12.10	5.94	0.17	55.75	7.47
SO_2 (ppb)	7.42	3.71	1.38	44.21	4.48
Temperature (°C)	11.20	9.86	-17.50	29.67	16.67

Table 3-3 Spearman Correlation coefficients across exposure metrics at concurrent case day

	$PM_{2.5}$	O_3	NO_2	SO_2	T_{mean}
$PM_{2.5}$	1.000				
O_3	0.313	1.000			
NO_2	0.442	-0.218	1.000		
SO_2	0.490	-0.116	0.607	1.000	
T_{mean}	0.402	0.714	-0.195	-0.163	1.000

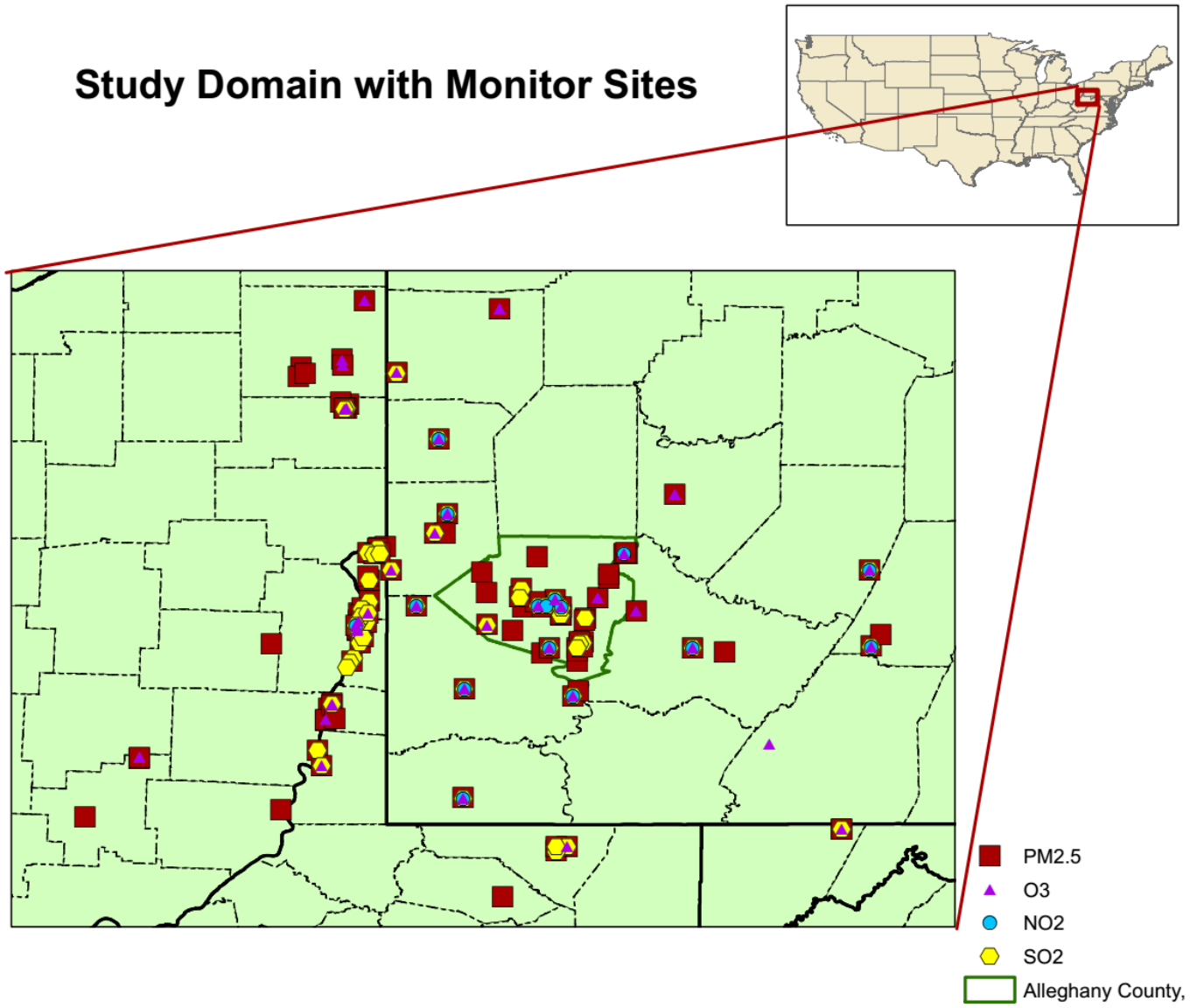

Figure 3-1 Study Domain with Monitor Sites

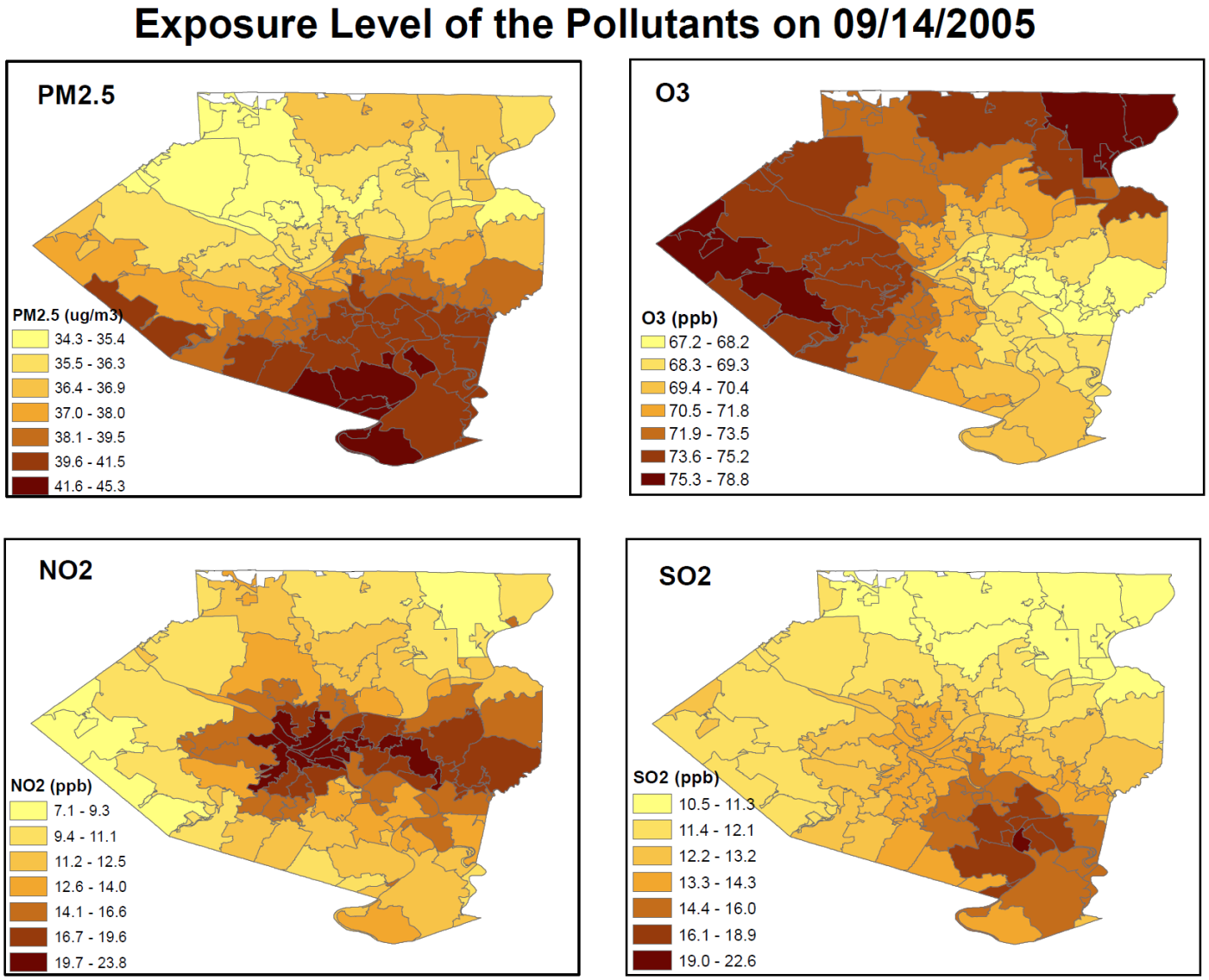

Figure 3-2 Spatial Variation of Exposure Levels of the Pollutants on a Specific Day, 09/14/2005

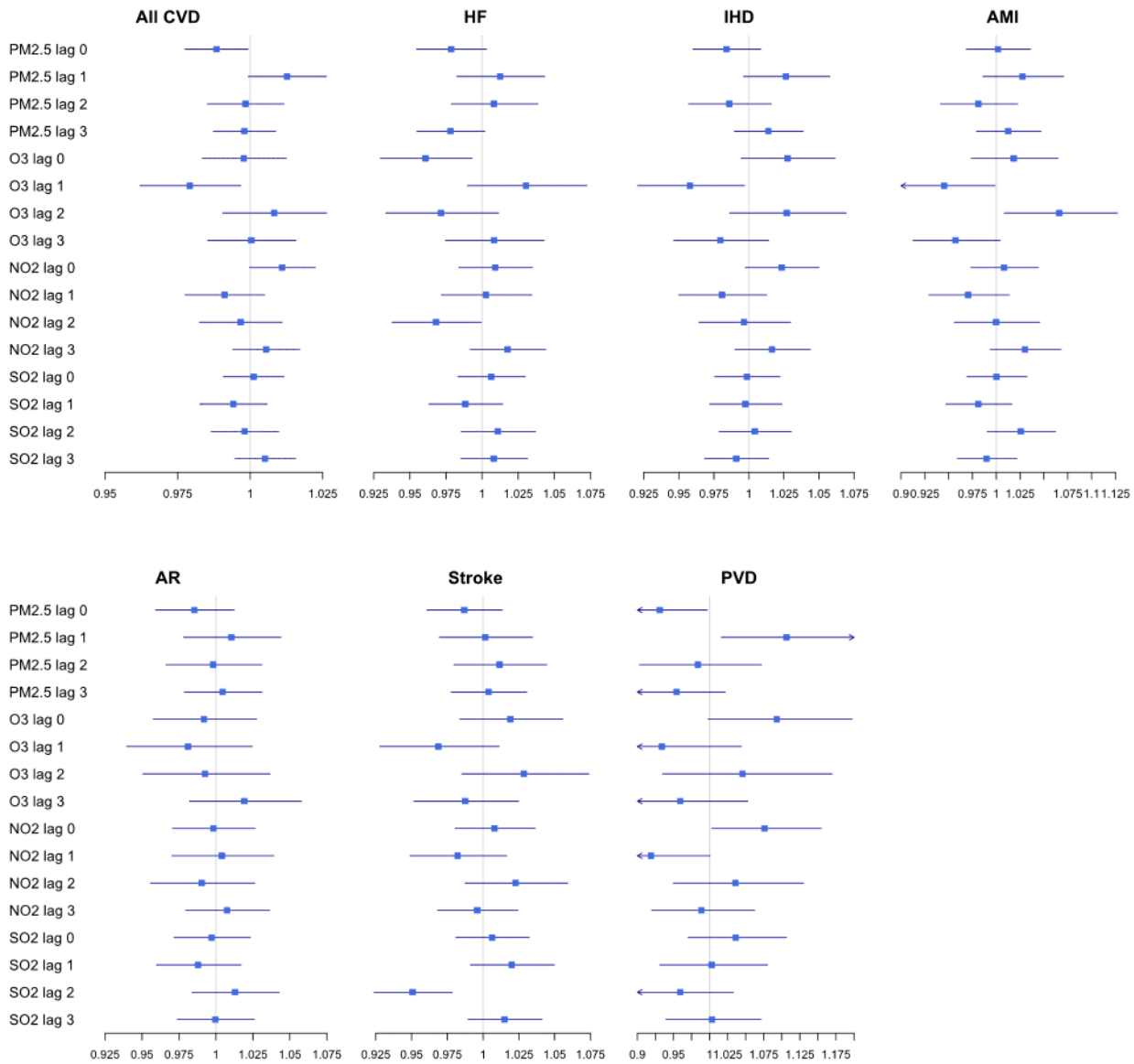

Figure 3-3 Multi-pollutant model of Criteria Pollutants and CVD Outcomes in Allegheny County, 1999 – 2011

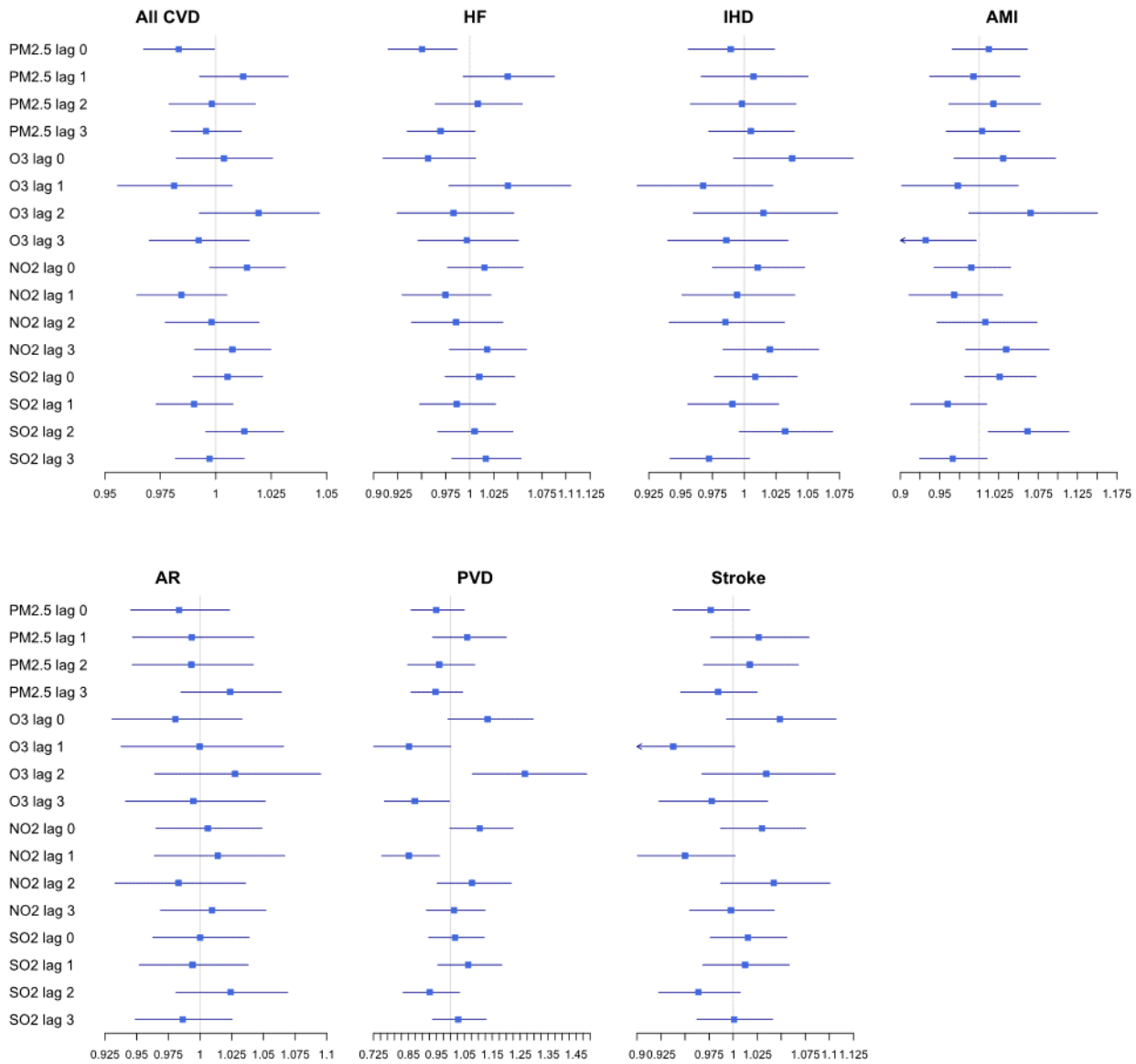

Figure 3-4 Multi-pollutant model of Criteria Pollutants and CVD Outcomes Among the Males in Allegheny County, 1999 – 2011

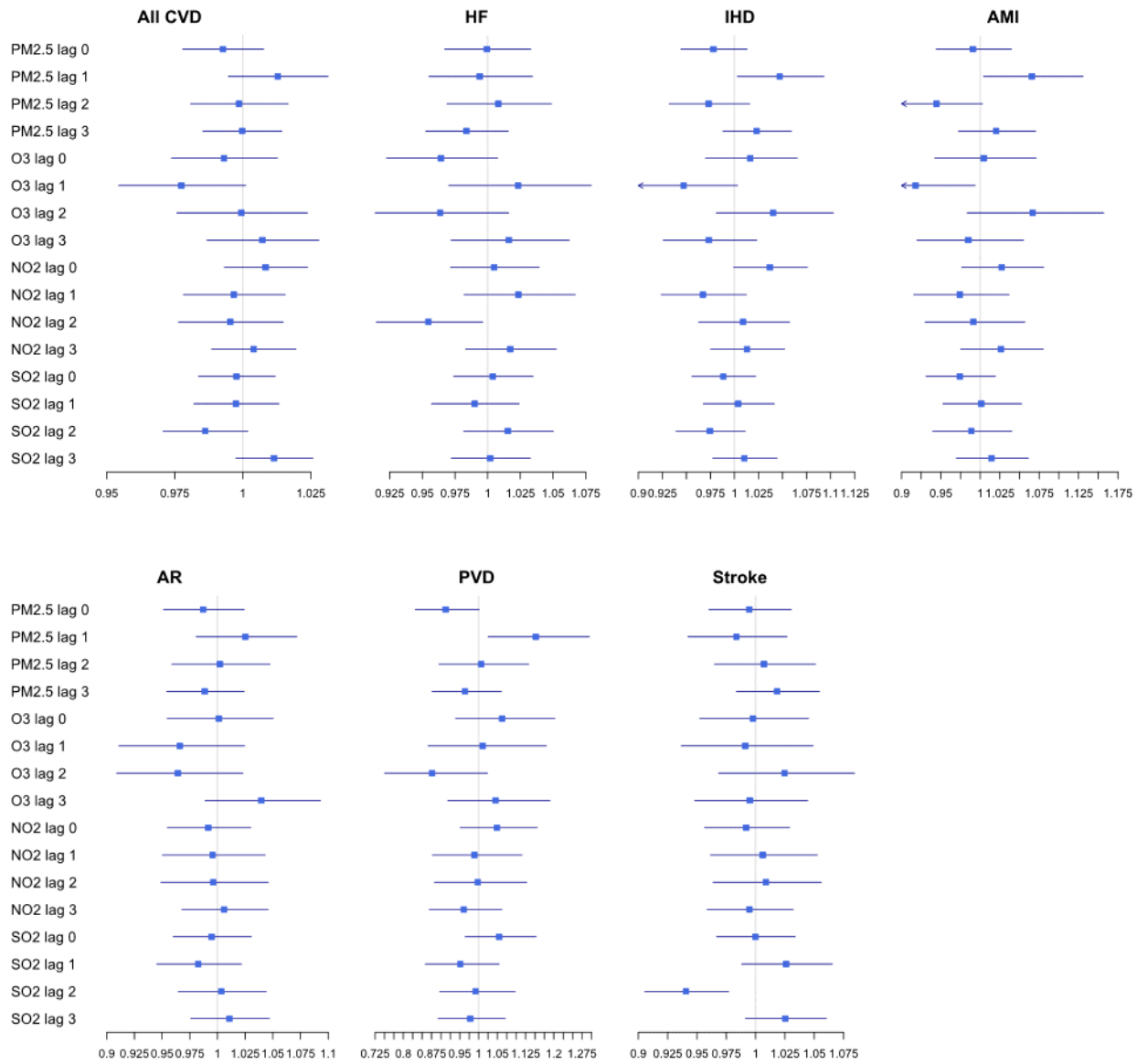

Figure 3-5 Multi-pollutant model of Criteria Pollutants and CVD Outcomes Among the Females in Allegheny County, 1999 – 2011

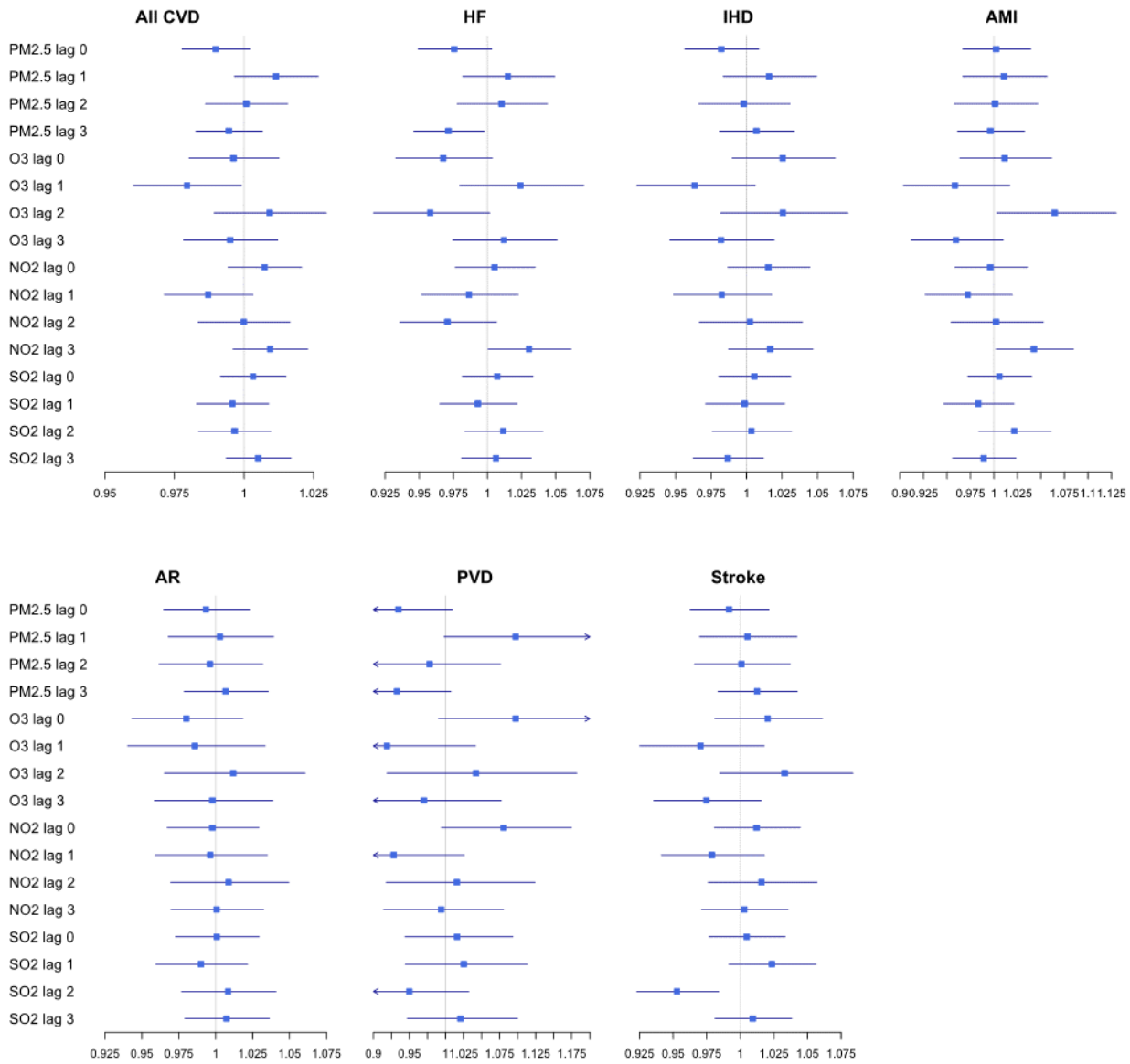

Figure 3-6 Multi-pollutant model of Criteria Pollutants and CVD Outcomes Among the White in Allegheny County, 1999 – 2011

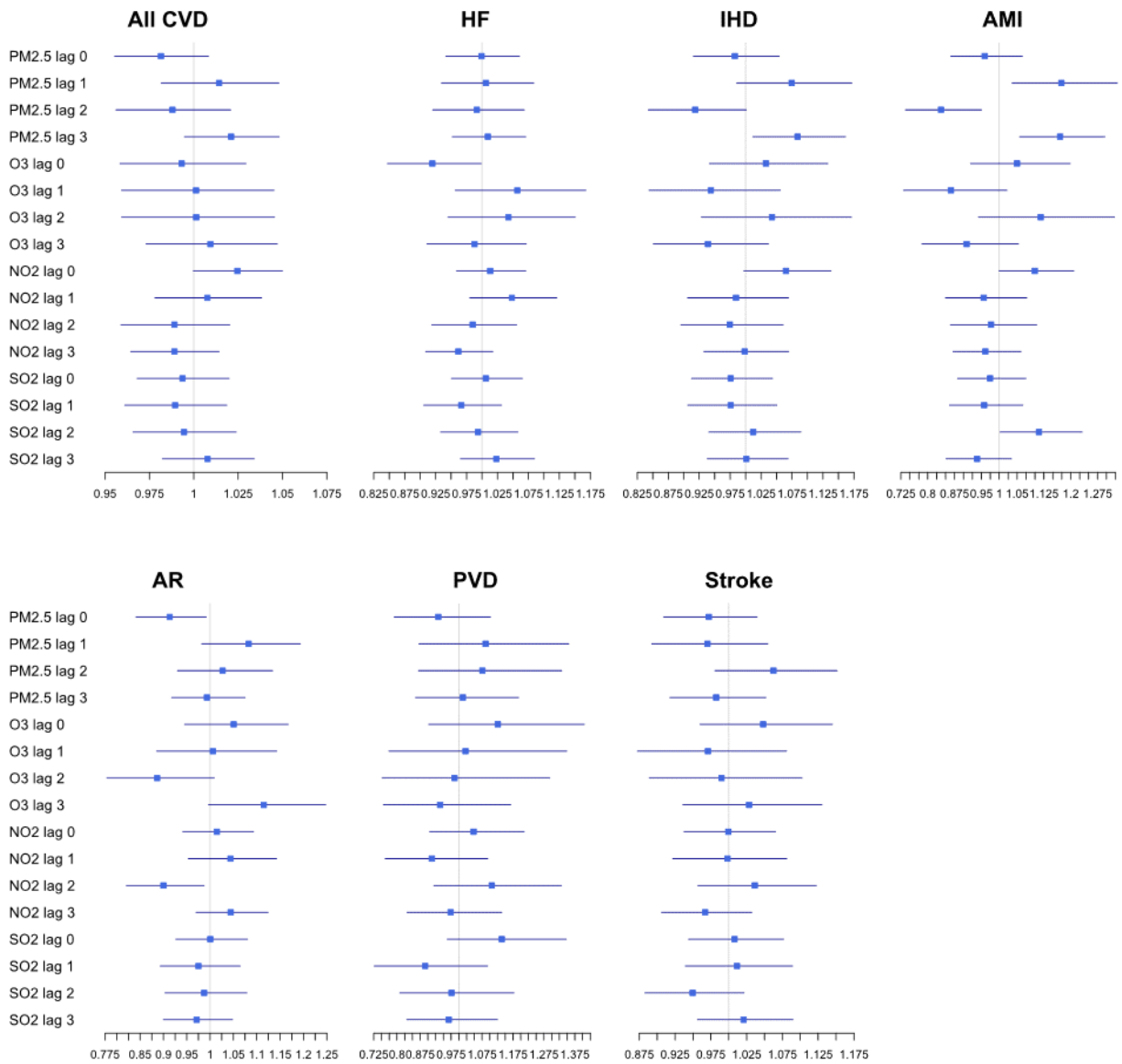

Figure 3-7 Multi-pollutant model of Criteria Pollutants and CVD Outcomes Among the Black in Allegheny County, 1999 – 2011

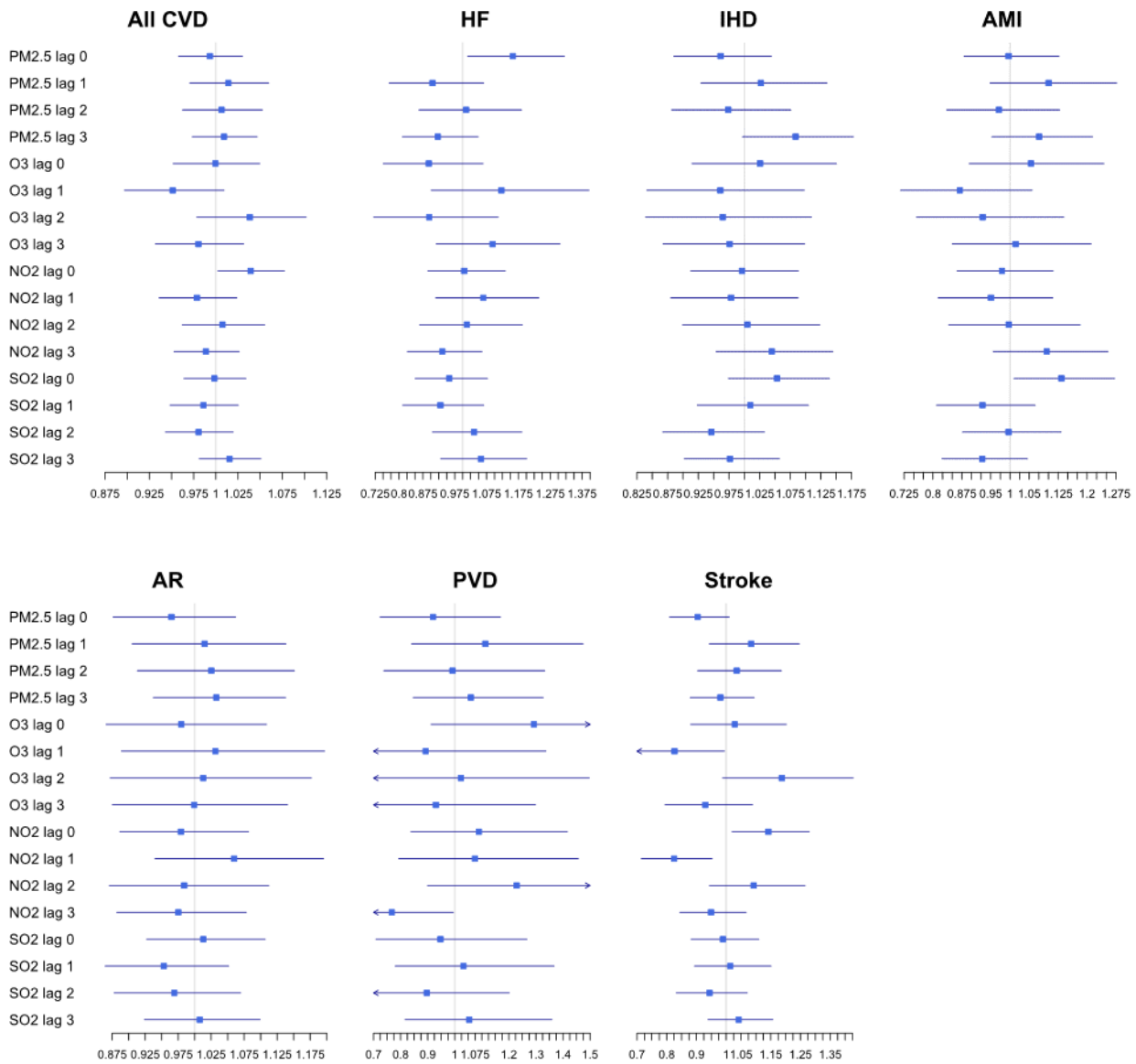

Figure 3-8 Multi-pollutant model of Criteria Pollutants and CVD Outcomes Among the People Aged 40 – 49 in Allegheny County, 1999 – 2011

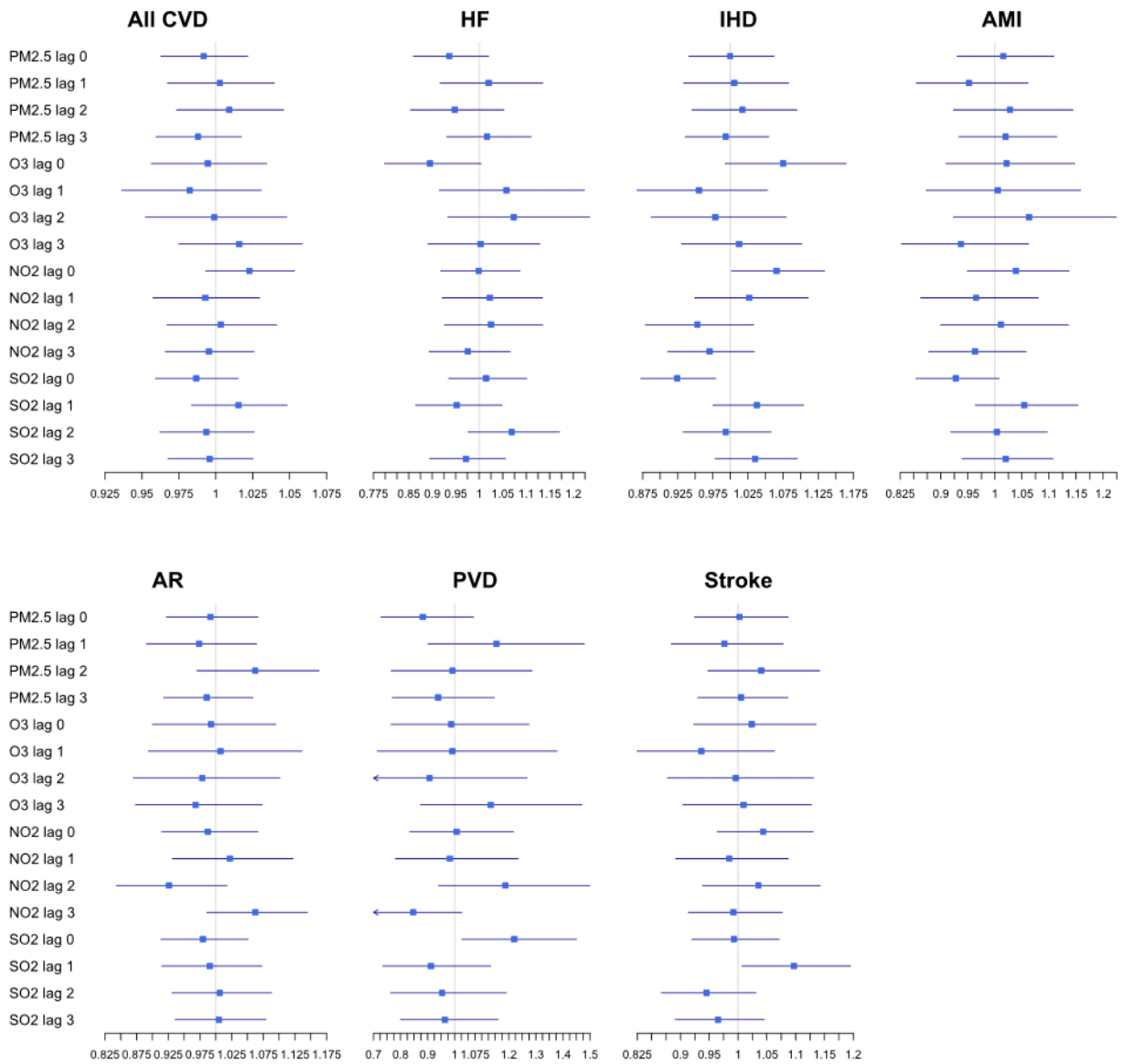

Figure 3-9 Multi-pollutant model of Criteria Pollutants and CVD Outcomes Among the People Aged 50 – 59 in Allegheny County, 1999 – 2011

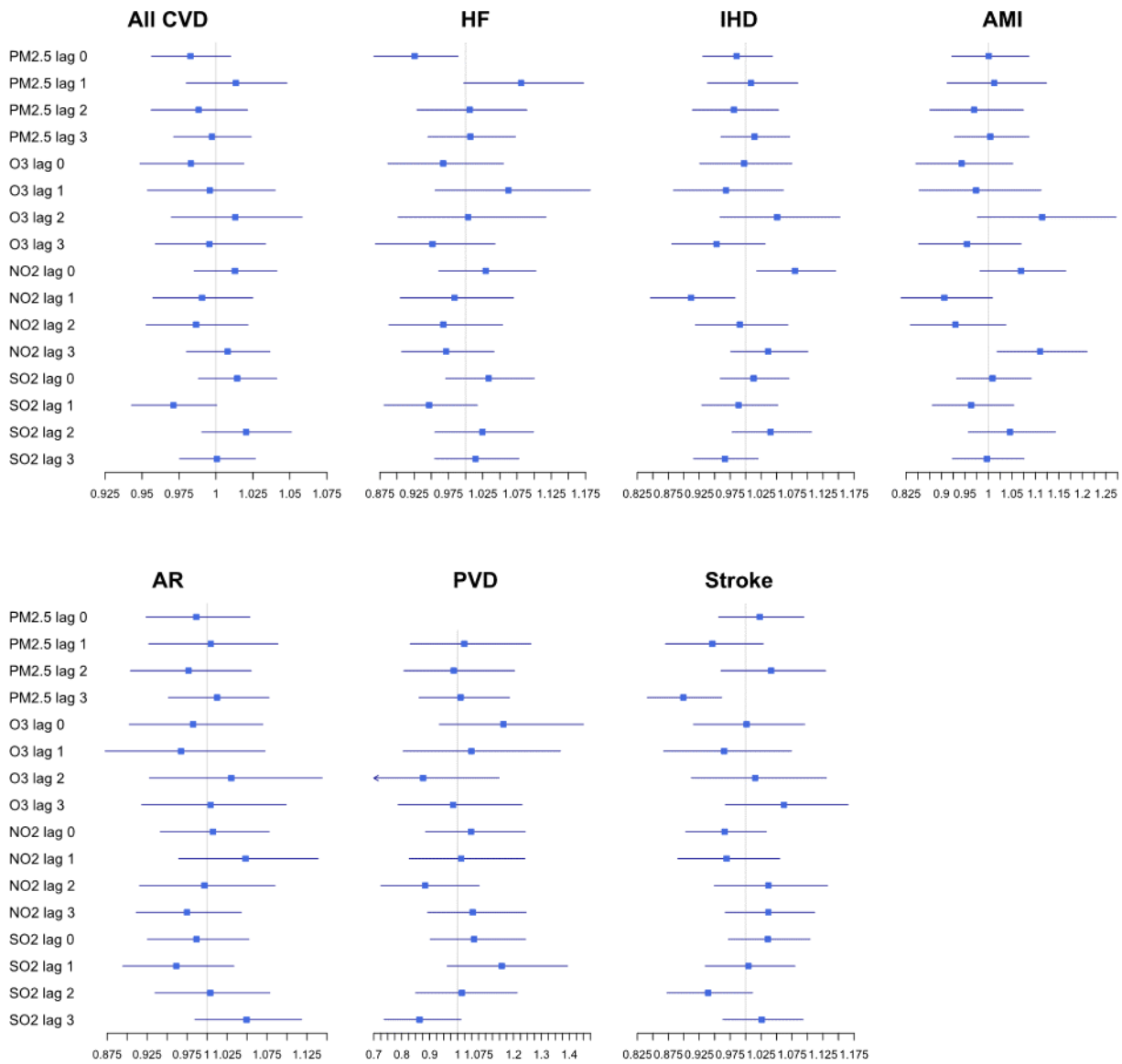

Figure 3-10 Multi-pollutant model of Criteria Pollutants and CVD Outcomes Among the People Aged 60 – 69 in Allegheny County, 1999 – 2011

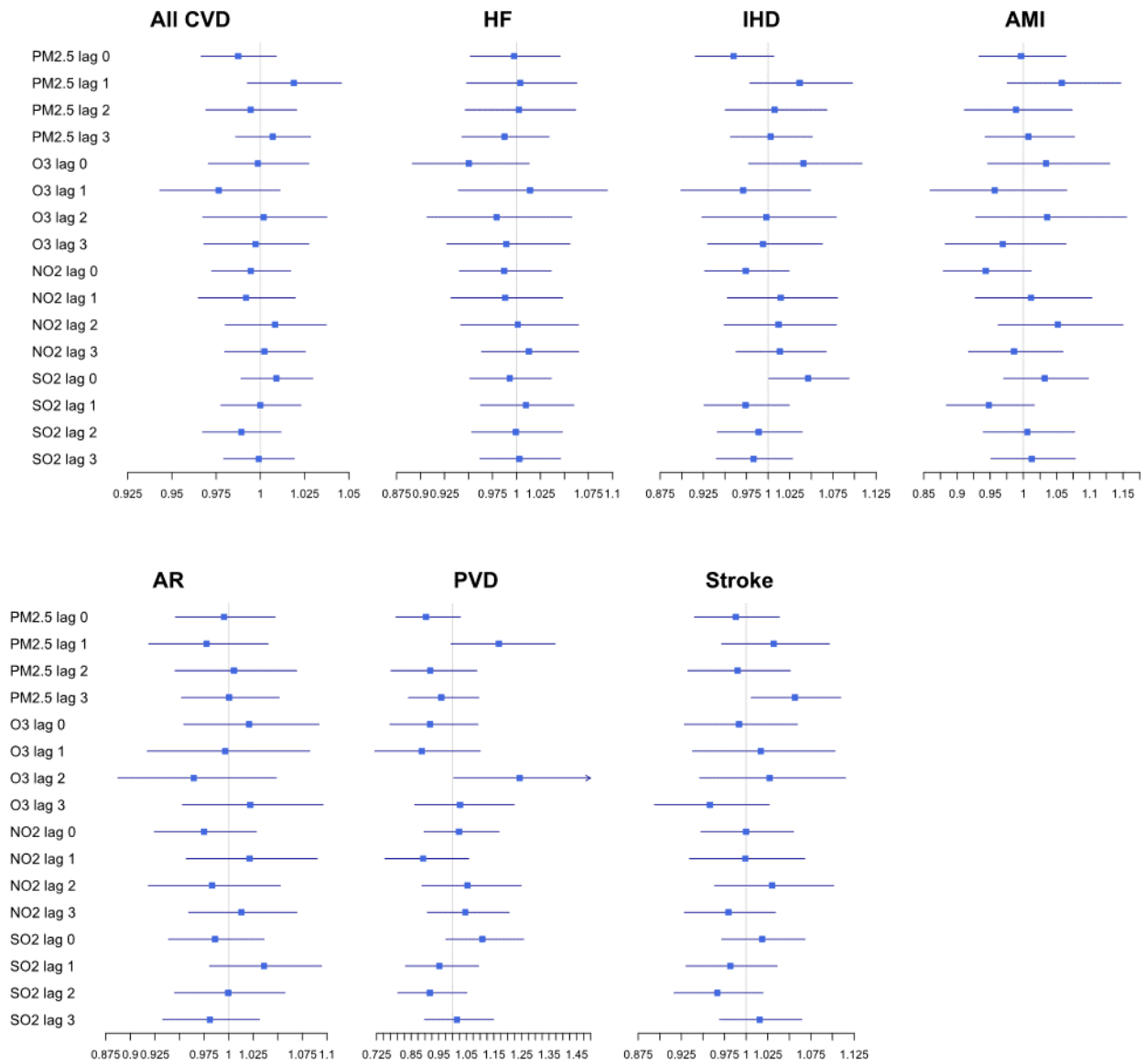

Figure 3-11 Multi-pollutant model of Criteria Pollutants and CVD Outcomes Among the People Aged 70 – 79 in Allegheny County, 1999 – 2011

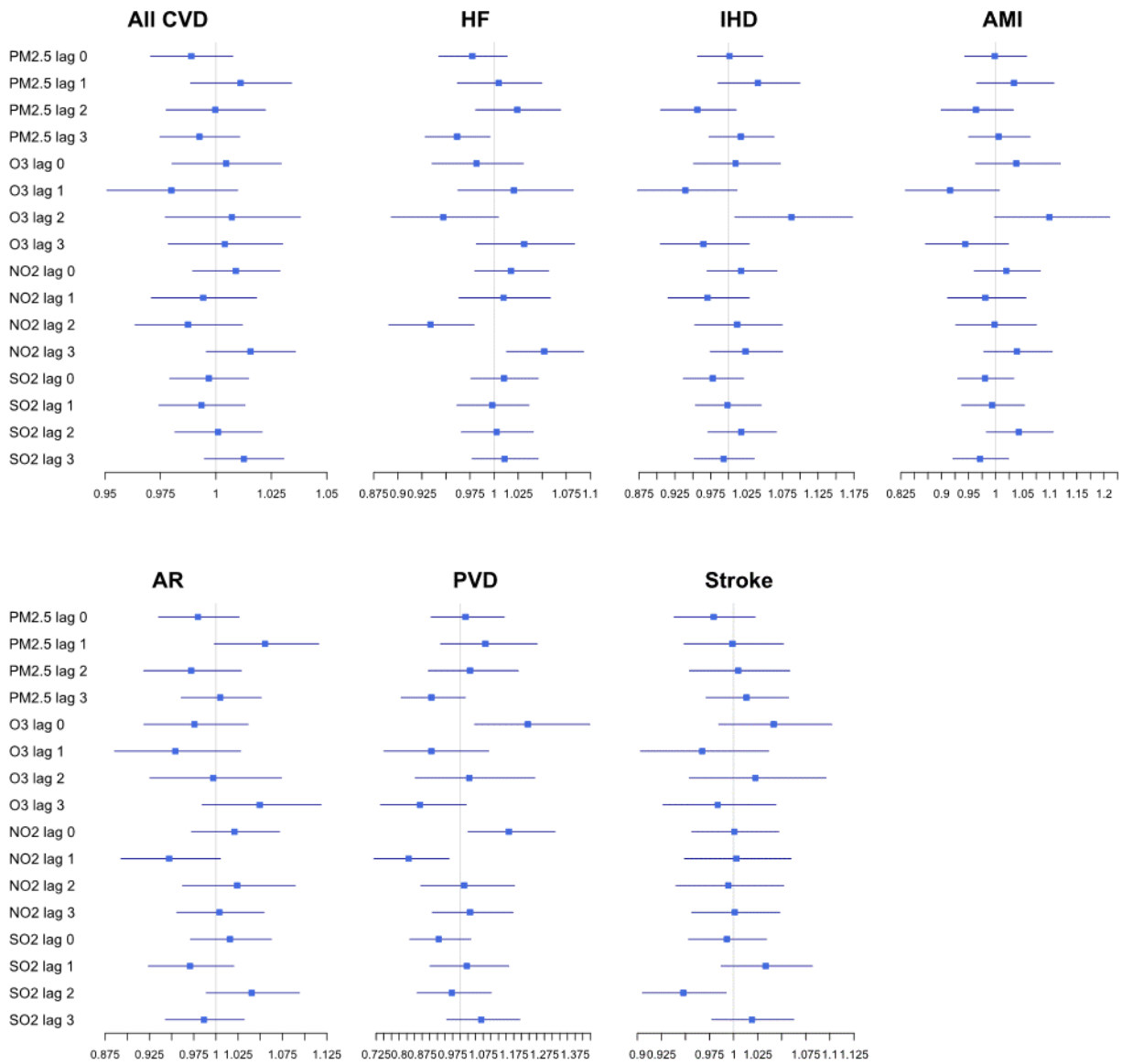

Figure 3-12 Multi-pollutant model of Criteria Pollutants and CVD Outcomes Among the People Aged 80 and older in Allegheny County, 1999 – 2011

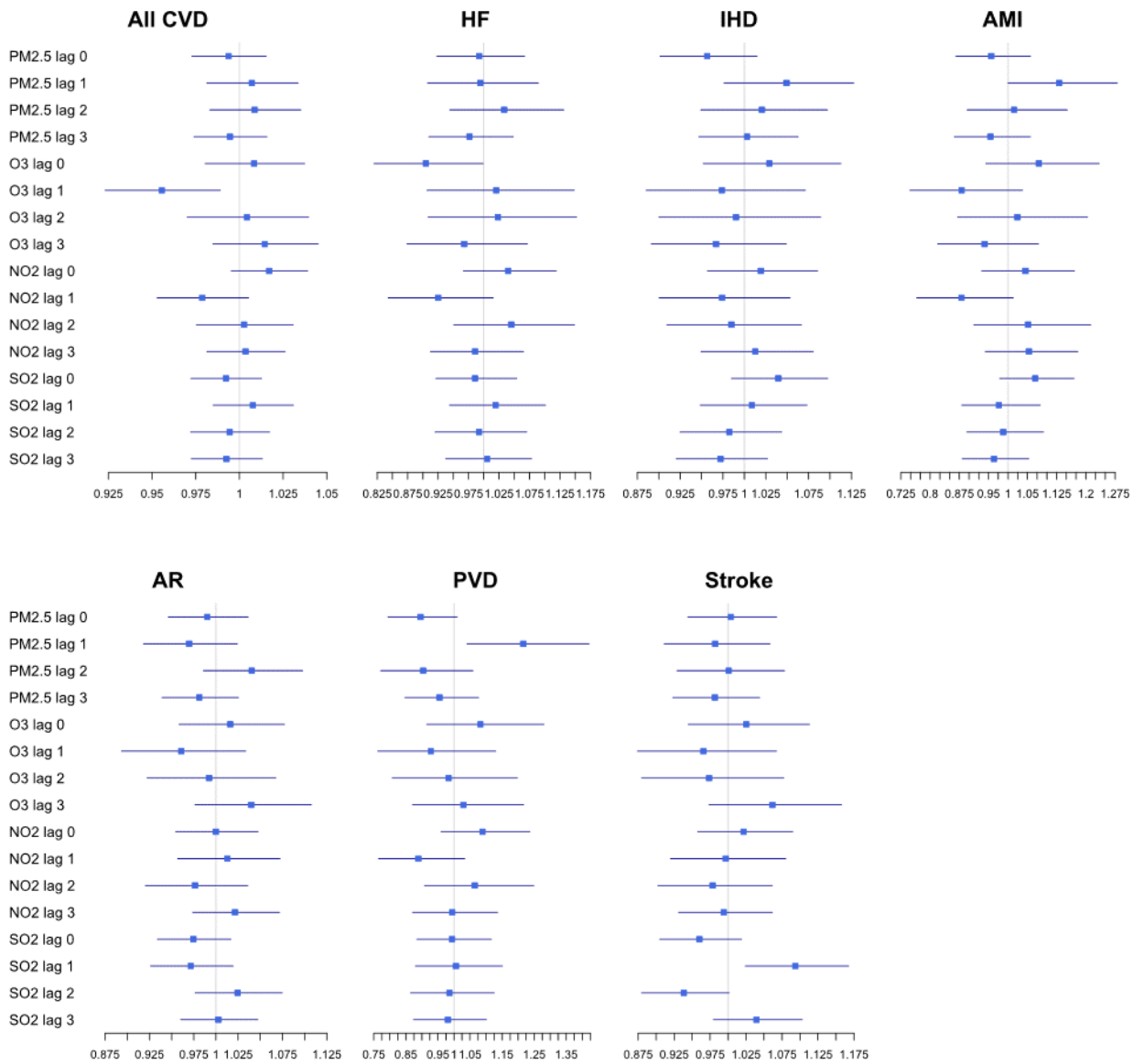

Figure 3-13 Multi-pollutant model of Criteria Pollutants and CVD Outcomes Among the Discharge in Allegheny County, 1999 – 2011

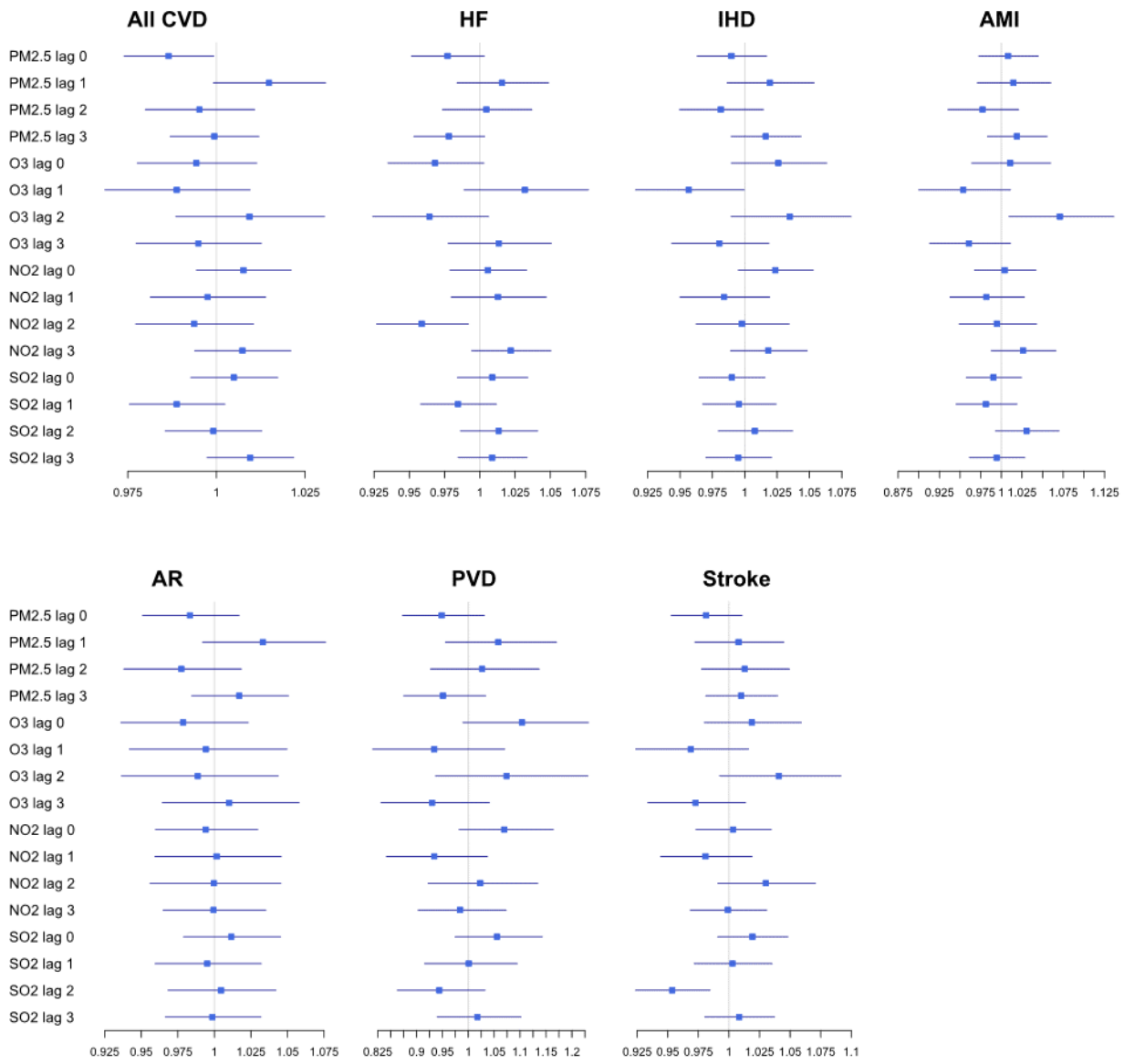

Figure 3-14 Multi-pollutant model of Criteria Pollutants and CVD Outcomes Among the In-patient in Allegheny County, 1999 – 2011

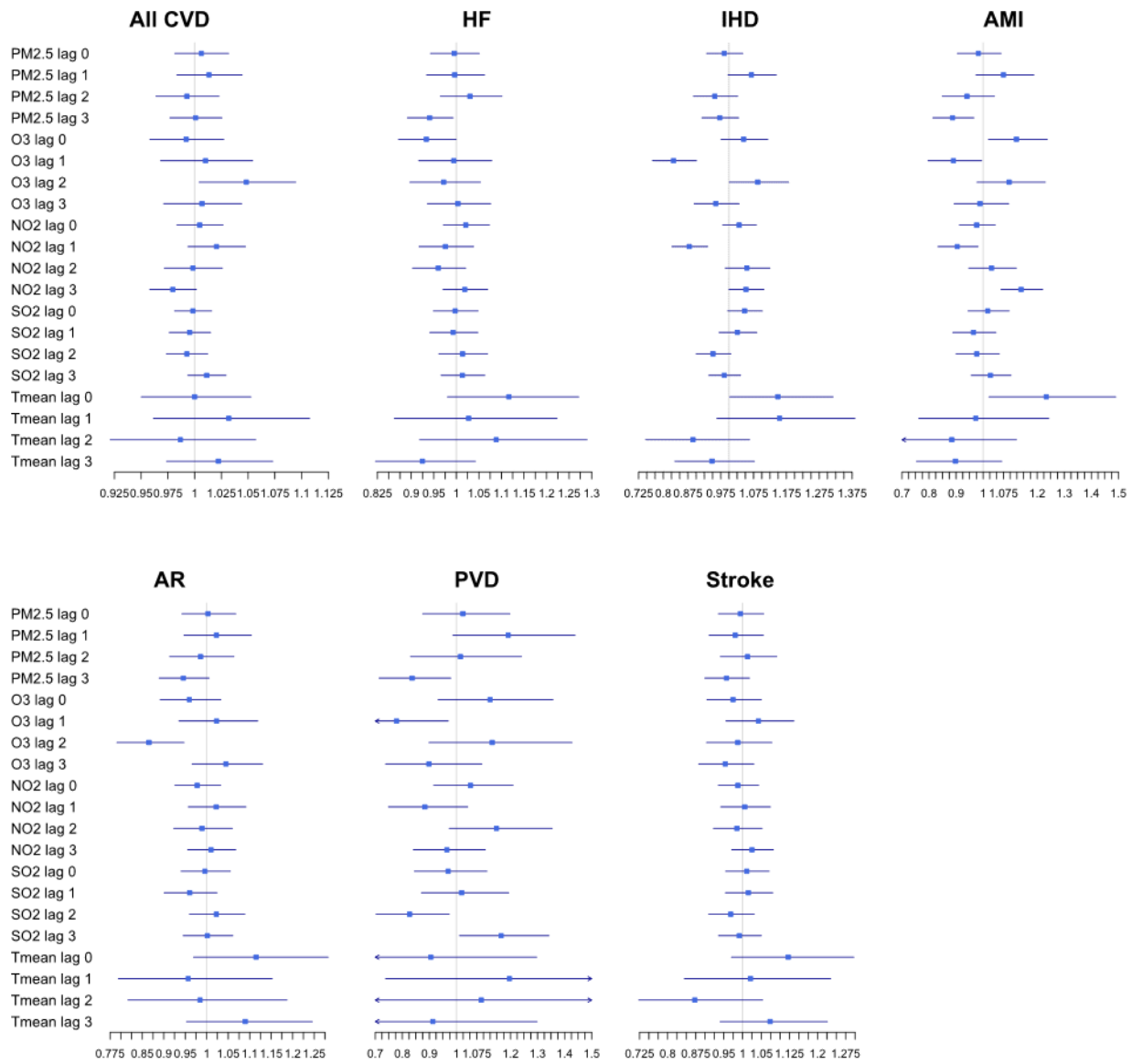

Figure 3-15 Multi-pollutant model of Criteria Pollutants and CVD Outcomes in the Spring in Allegheny County, 1999 – 2011

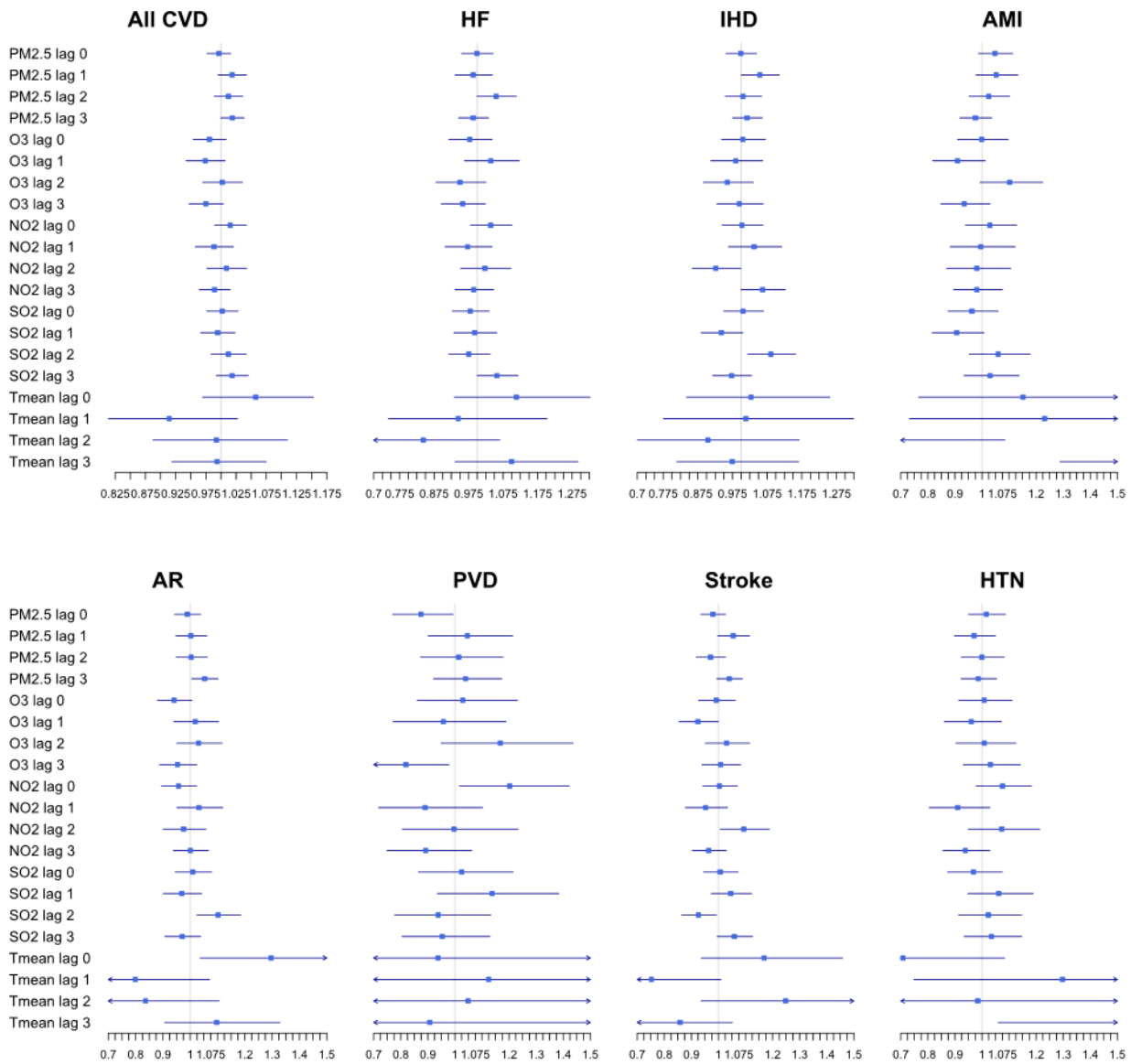

Figure 3-16 Multi-pollutant model of Criteria Pollutants and CVD Outcomes in the Summer in Allegheny County, 1999 – 2011

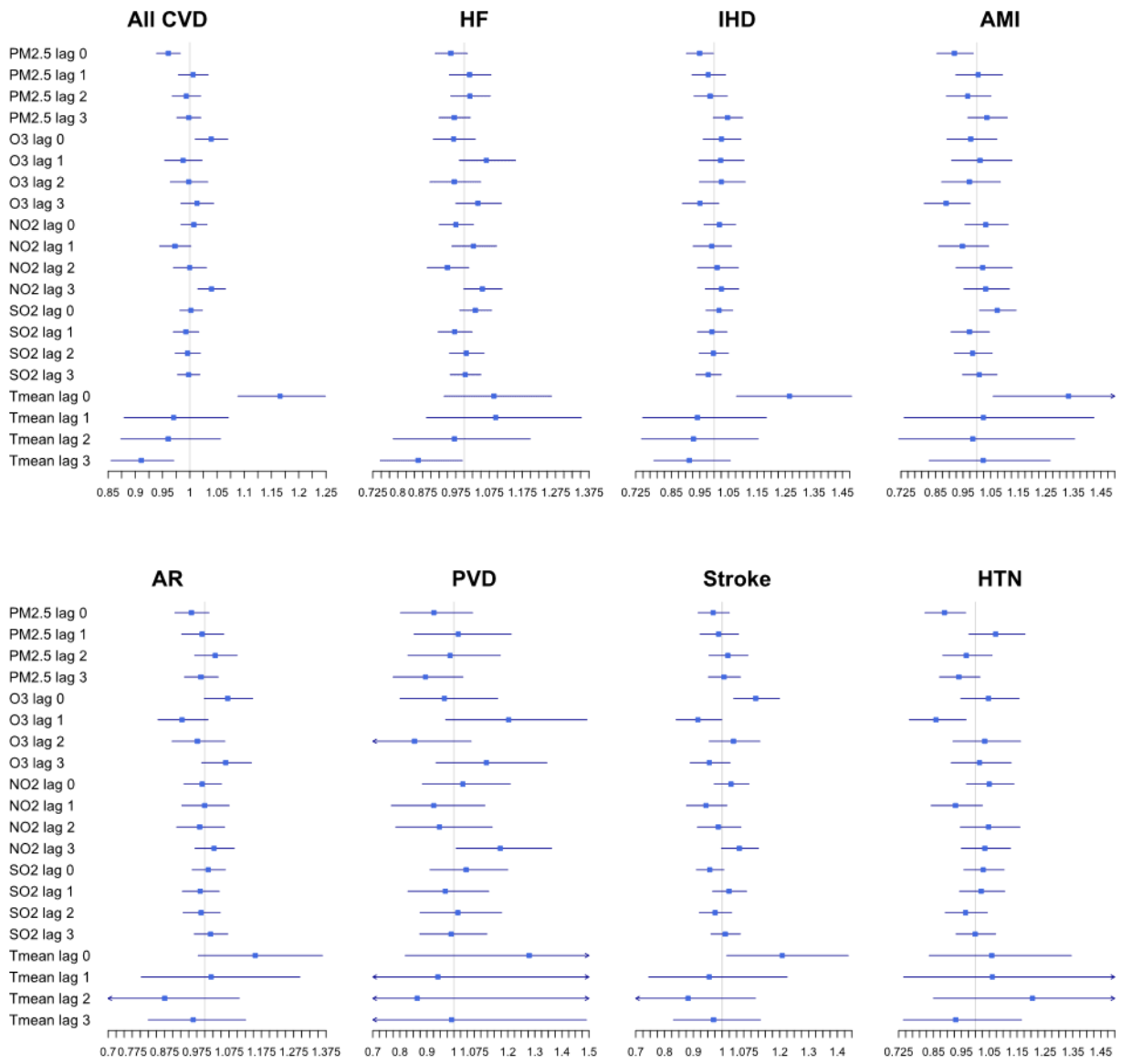

Figure 3-17 Multi-pollutant model of Criteria Pollutants and CVD Outcomes in the Fall in Allegheny County, 1999 – 2011

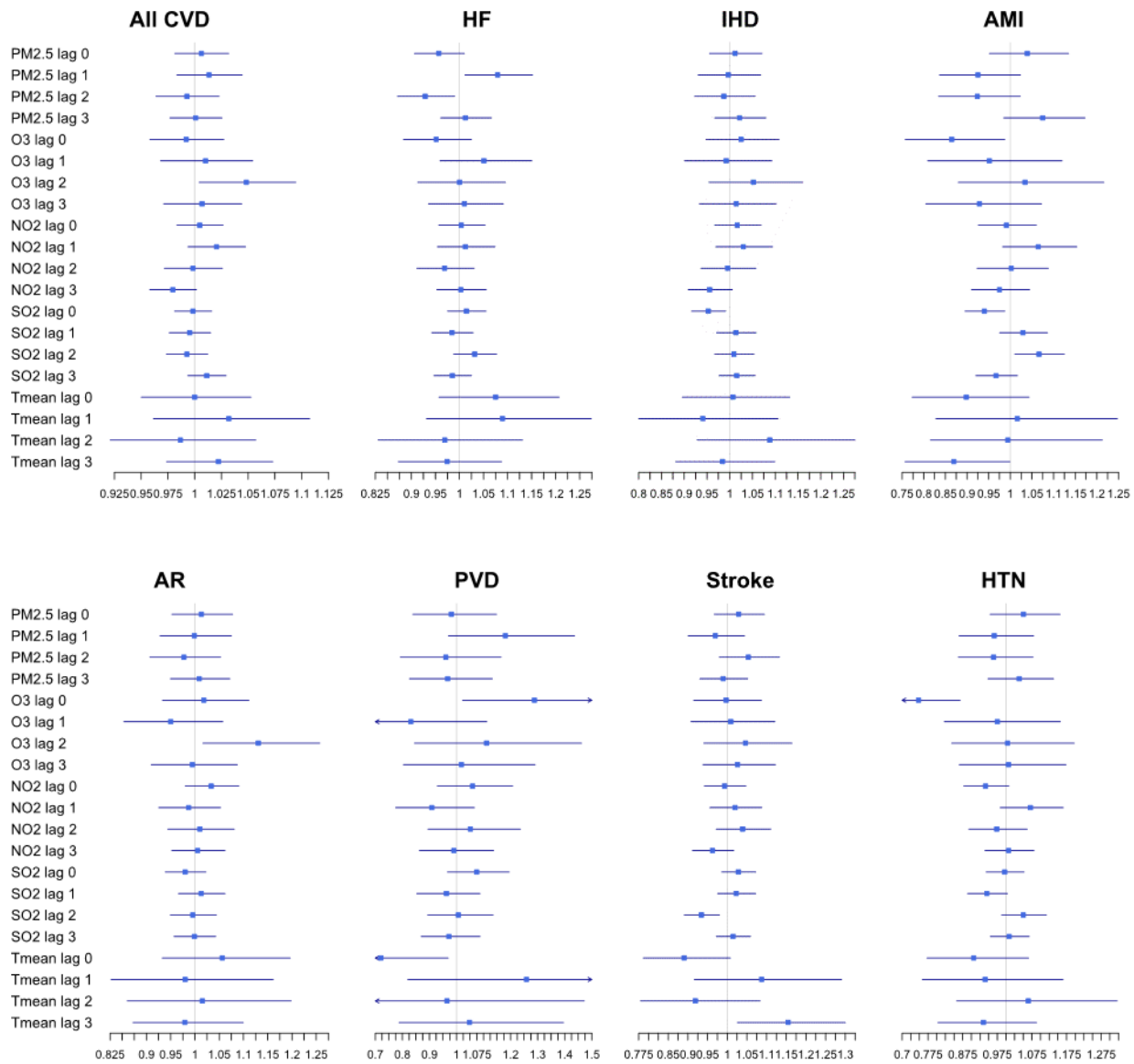

Figure 3-18 Multi-pollutant model of Criteria Pollutants and CVD Outcomes in the Winter in Allegheny County, 1999 – 2011

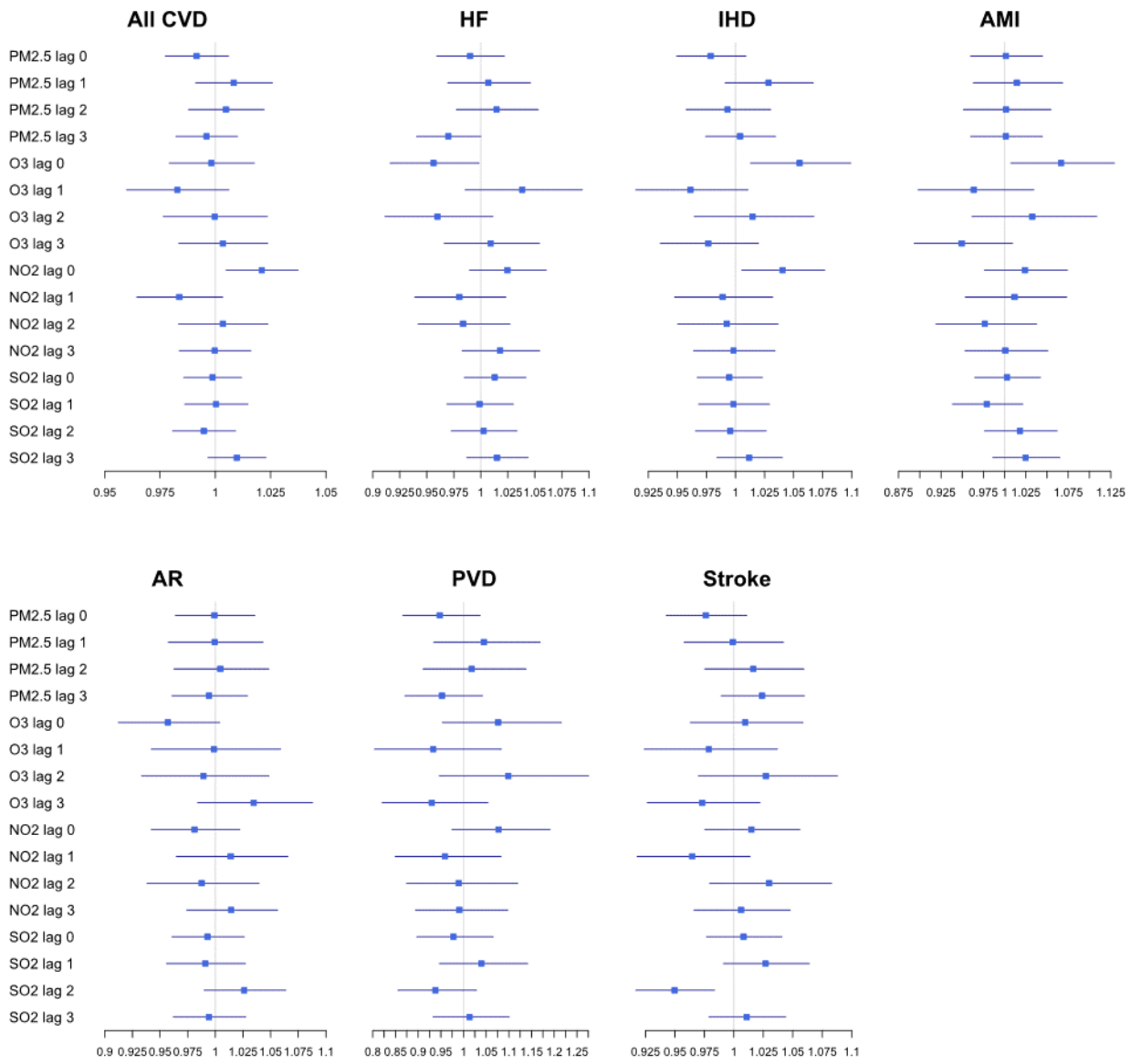

Figure 3-19 Multi-pollutant Model of Emergency Department visits of Cardiovascular Disease in Allegheny County, 1999 – 2005

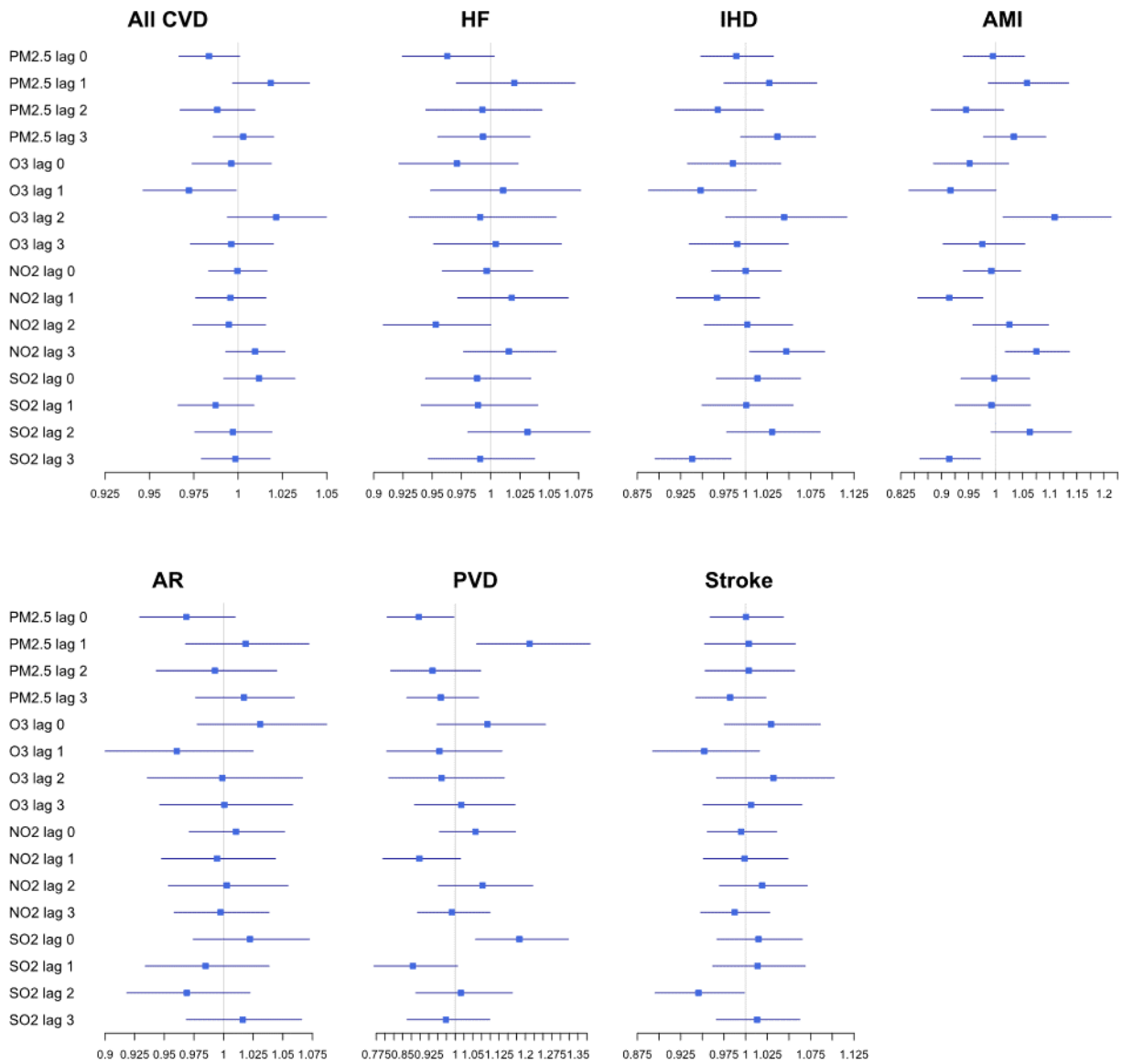

Figure 3-20 Multi-pollutant Model of Emergency Department visits of Cardiovascular Disease in Allegheny County, 2006 – 2011

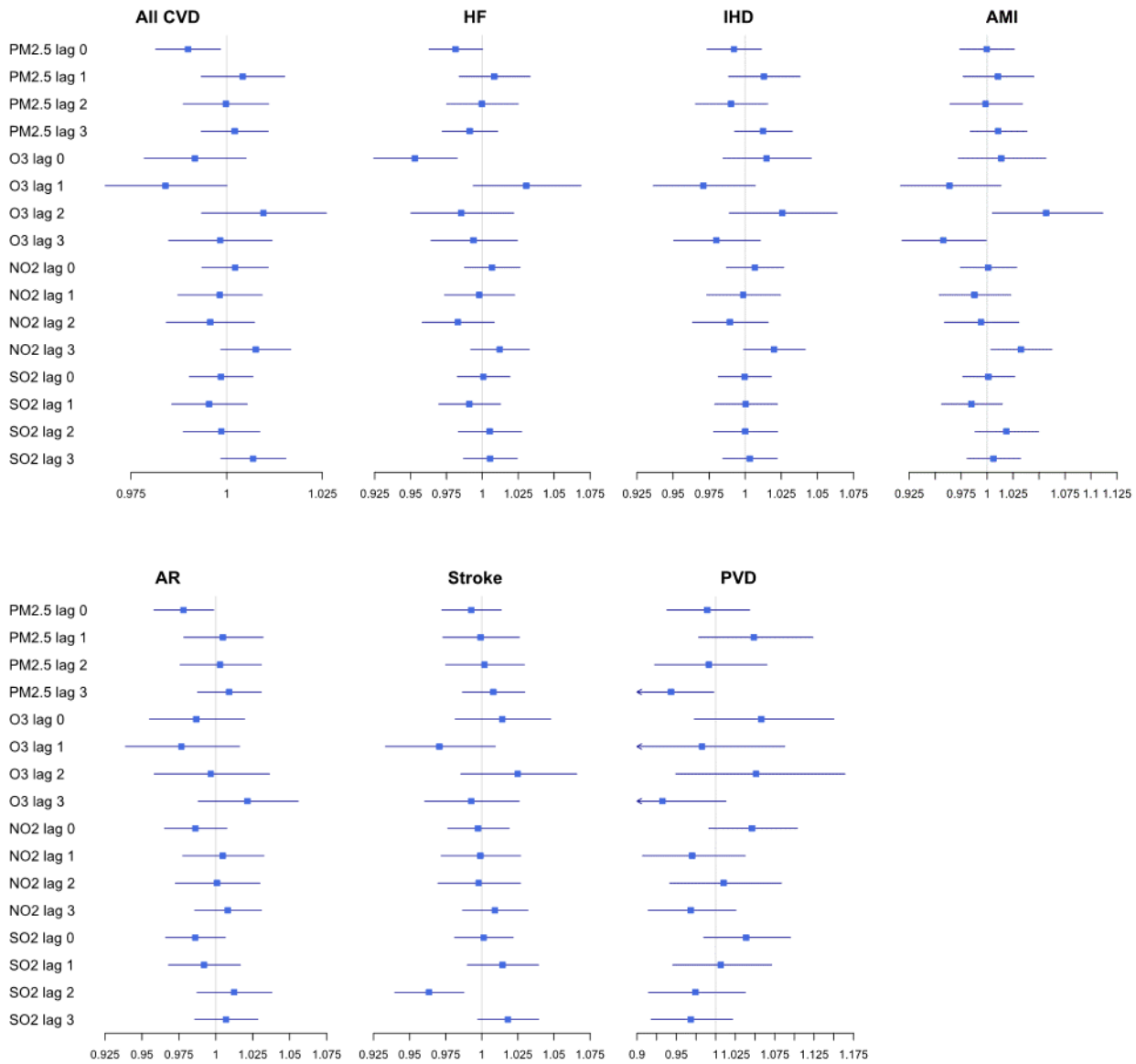

Figure S 3-1 Single Pollutant model of Criteria Pollutants and Cardiovascular Emergency Room Visits in Allegheny County, 1999 – 2011

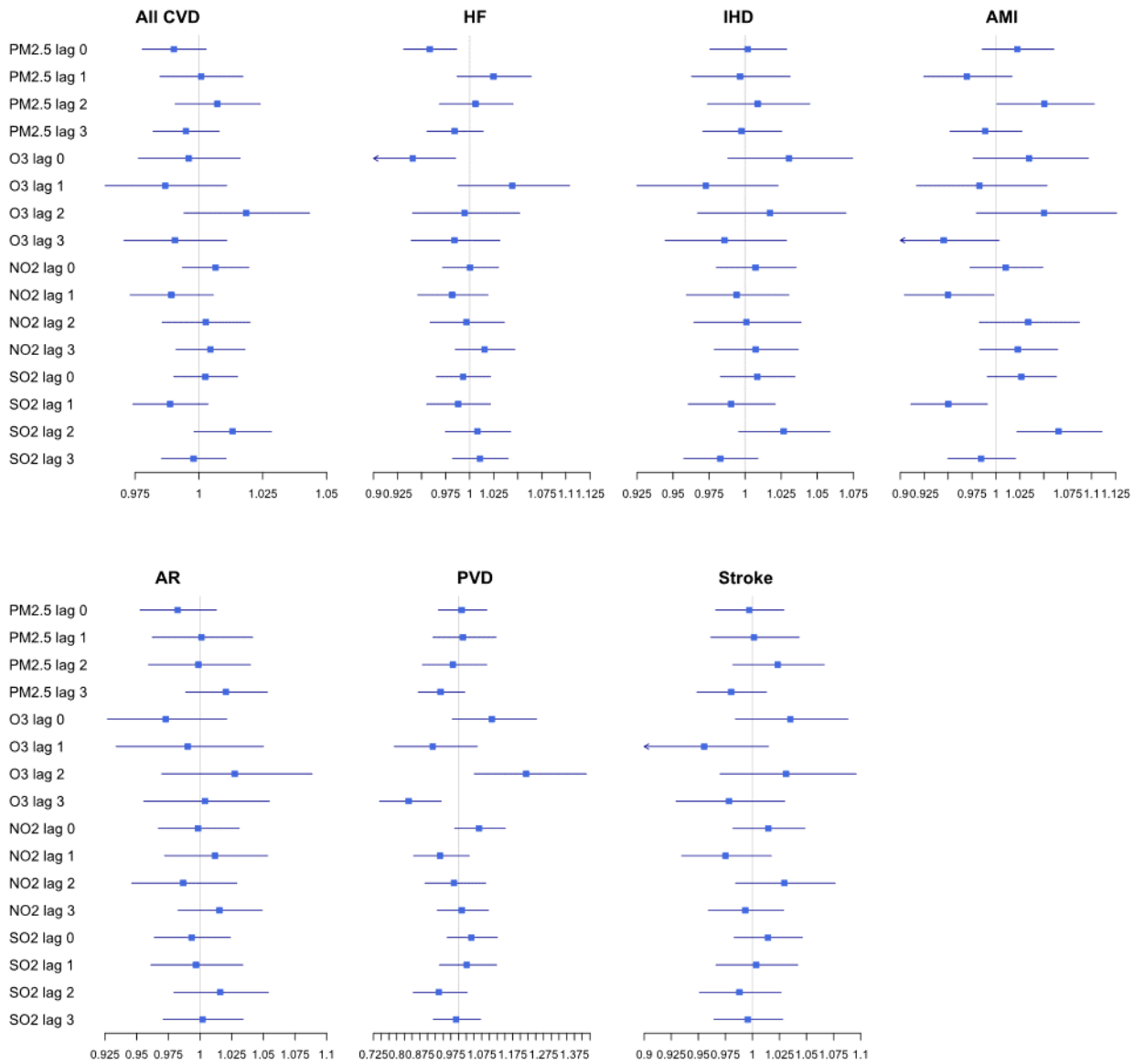

Figure S 3-2 Single Pollutant model of Criteria Pollutants and Cardiovascular Emergency Room Visits Among the Males in Allegheny County, 1999 – 2011

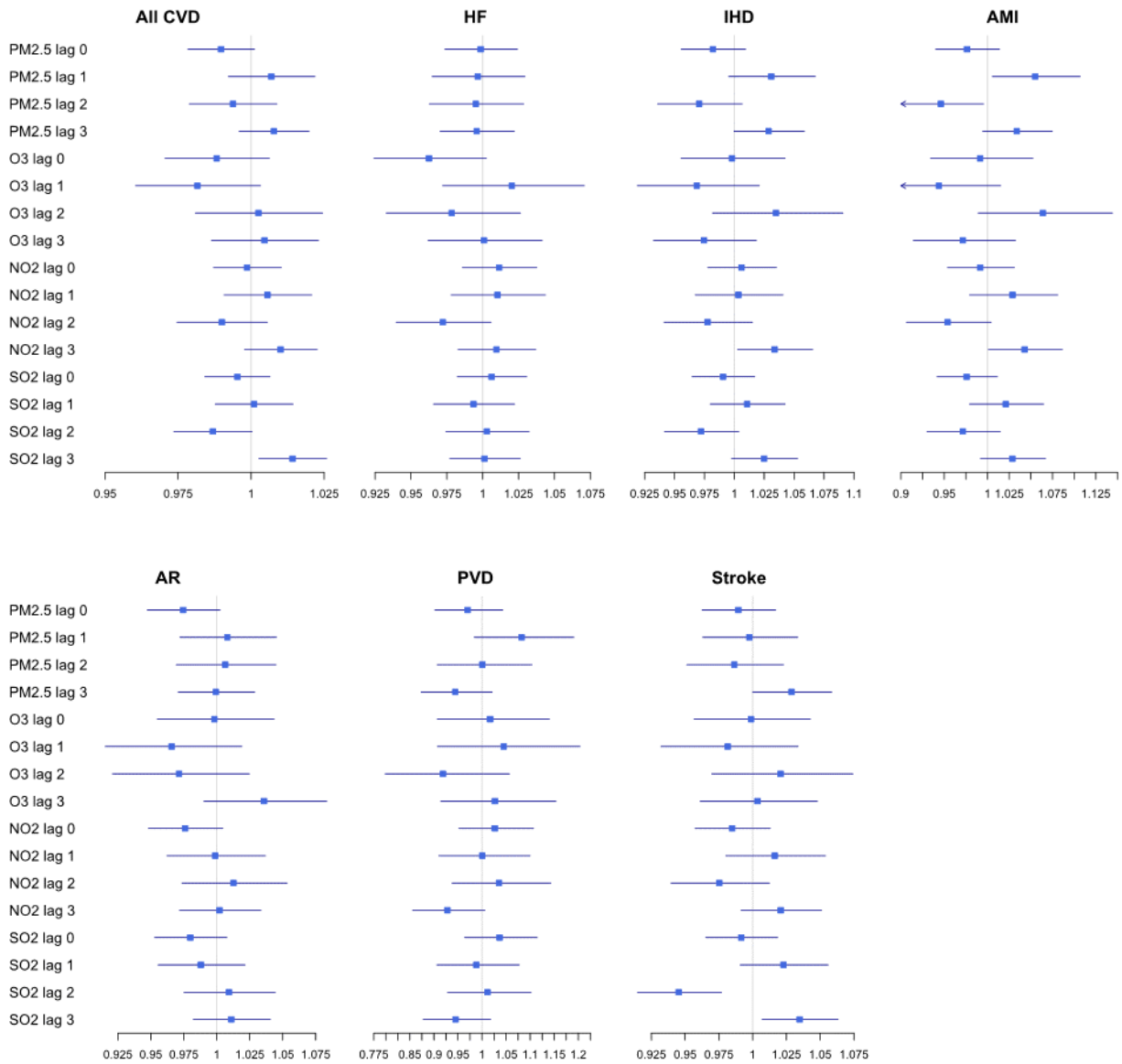

Figure S 3-3 Single pollutant model of Criteria Pollutants and Cardiovascular Emergency Room Visits Among the Females in Allegheny County, 1999 – 2011

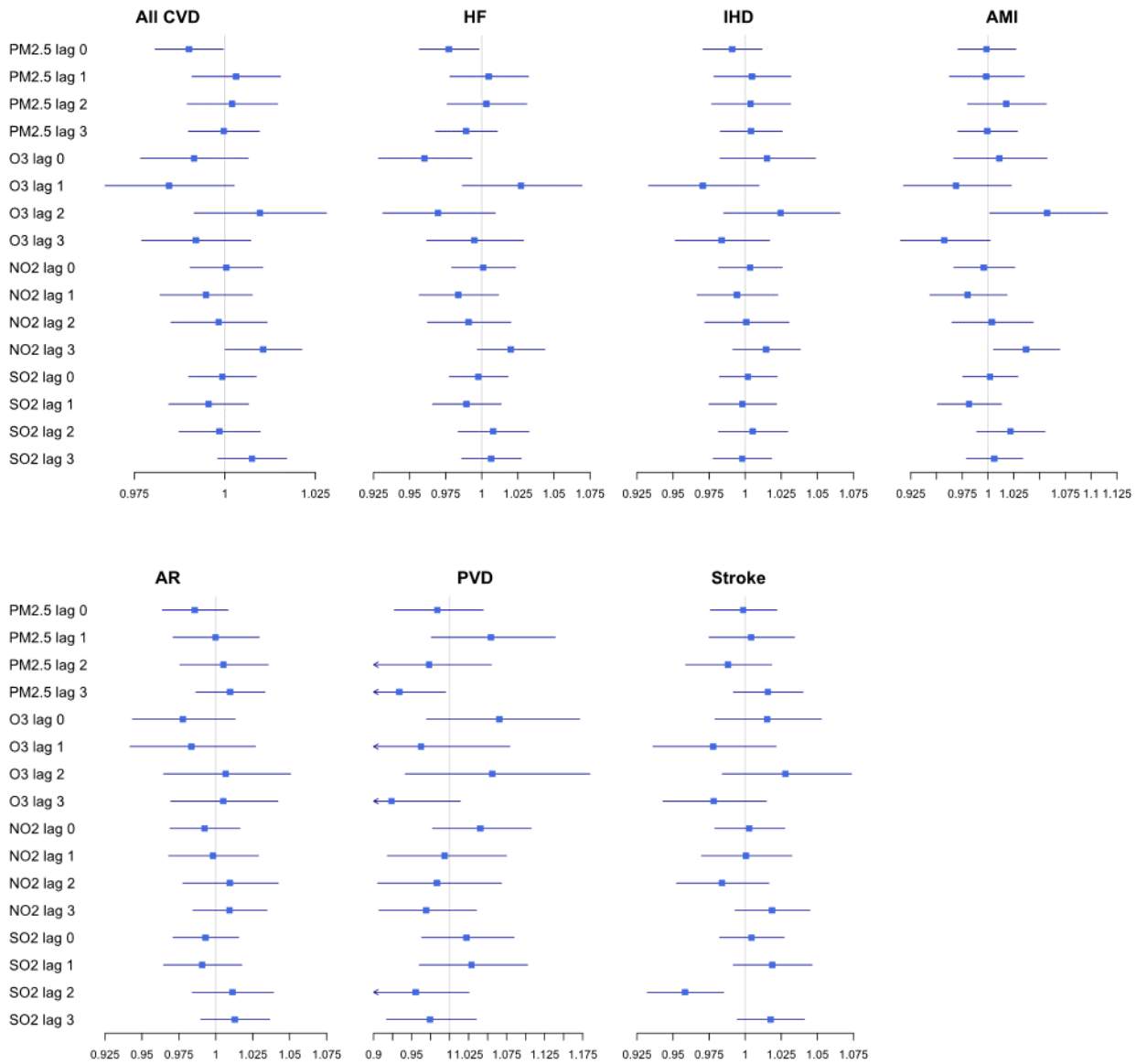

Figure S 3-4 Single Pollutant model of Criteria Pollutants and Cardiovascular Emergency Room Visits Among the White in Allegheny County, 1999 – 2011

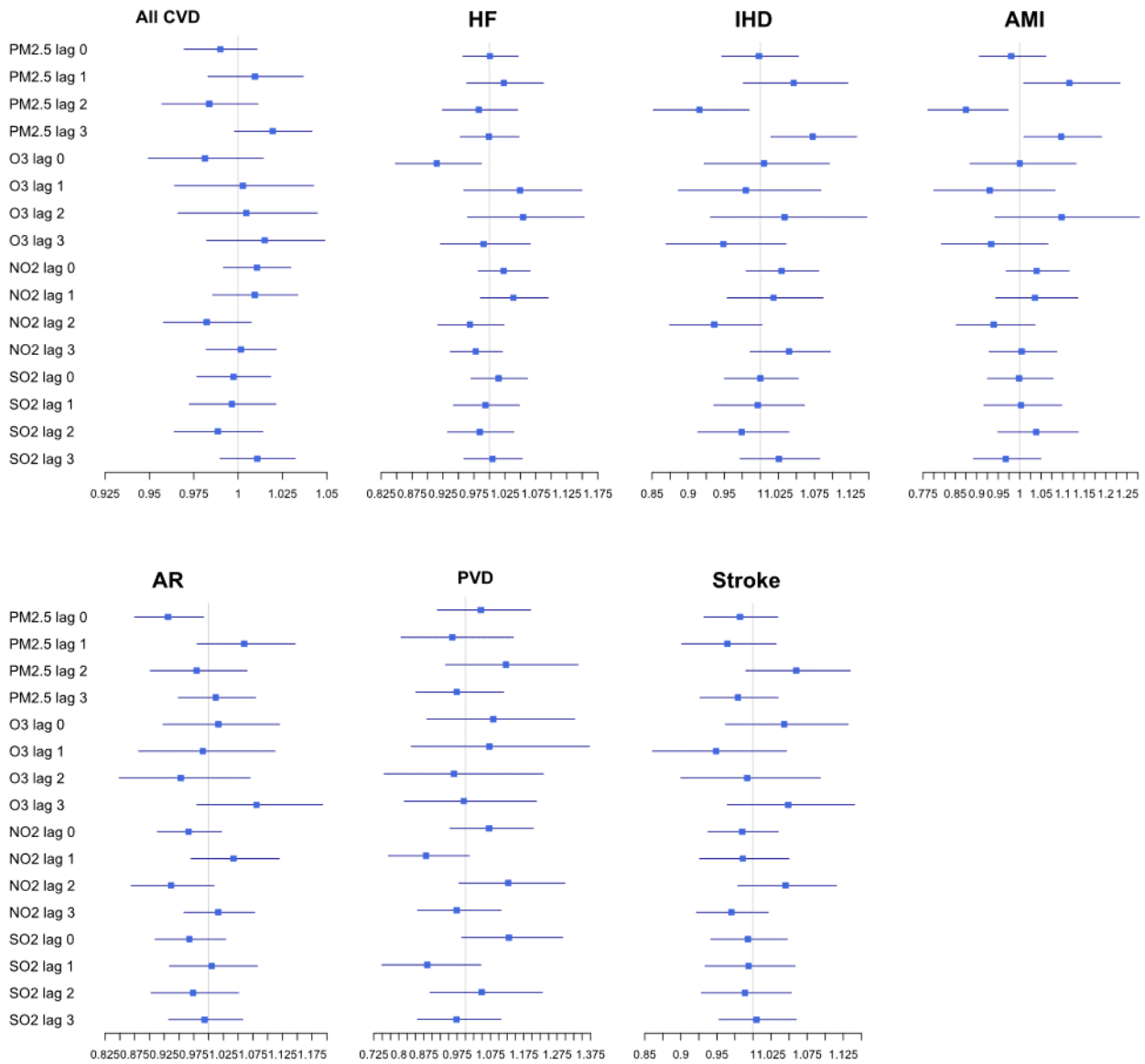

Figure S 3-5 Single Pollutant model of Criteria Pollutants and Cardiovascular Emergency Room Visits Among the Black in Allegheny County, 1999 – 2011

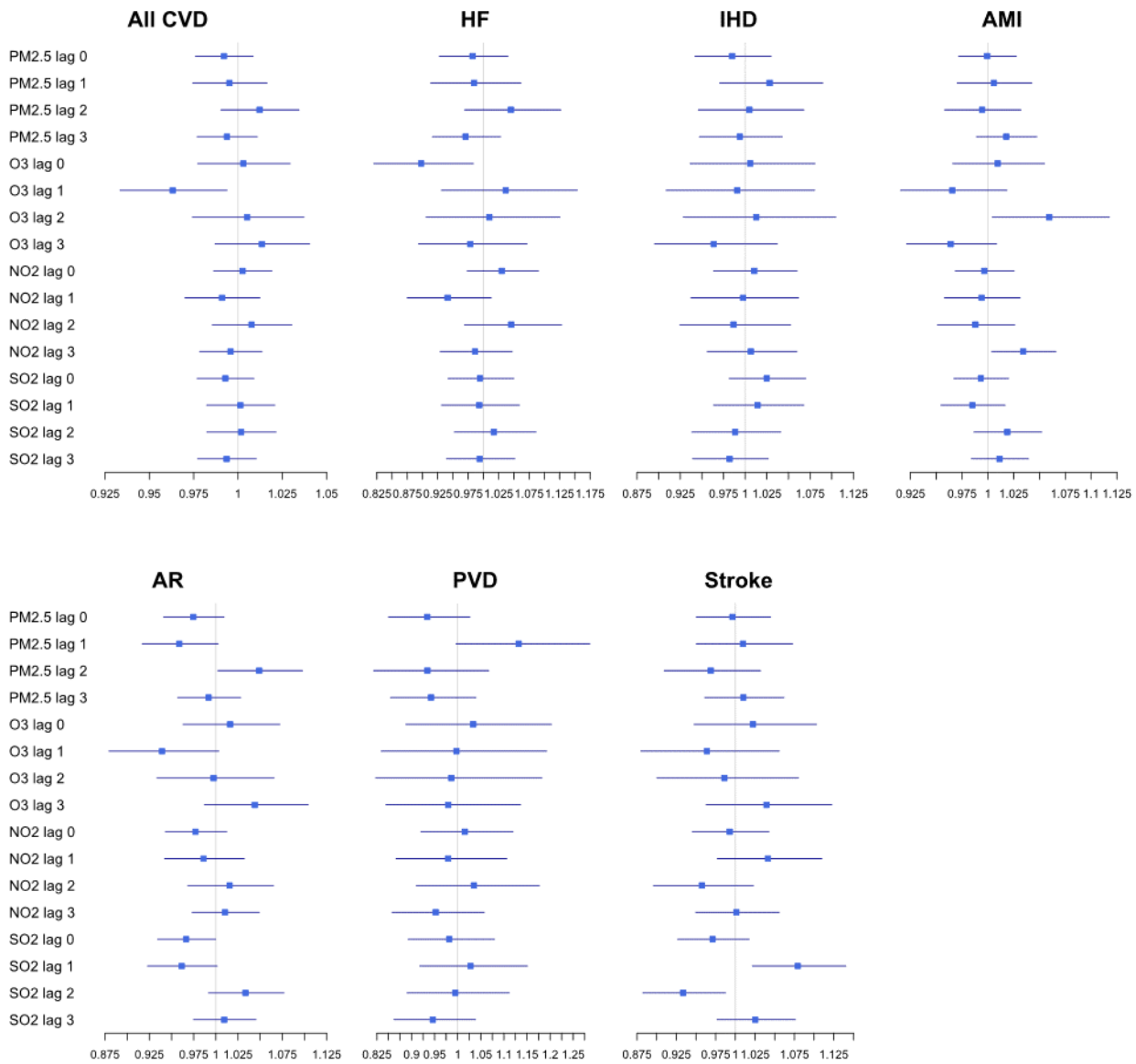

Figure S 3-6 Single Pollutant model of Criteria Pollutants and Cardiovascular Emergency Room Visits Among the Discharge in Allegheny County, 1999 – 2011

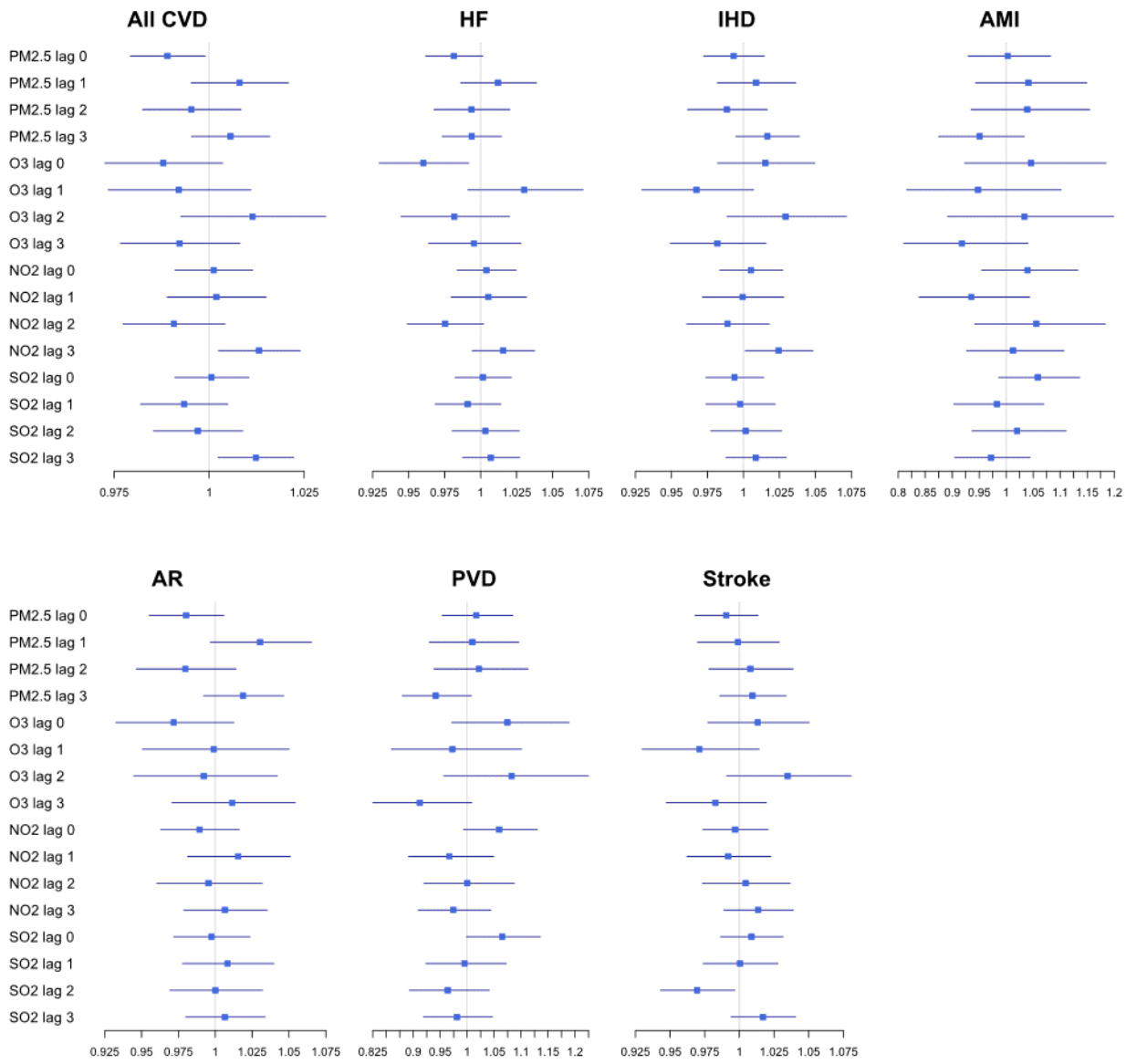

Figure S 3-7 Single Pollutant model of Criteria Pollutants and Cardiovascular Emergency Room Visits Among the In-patient in Allegheny County, 1999 – 2011

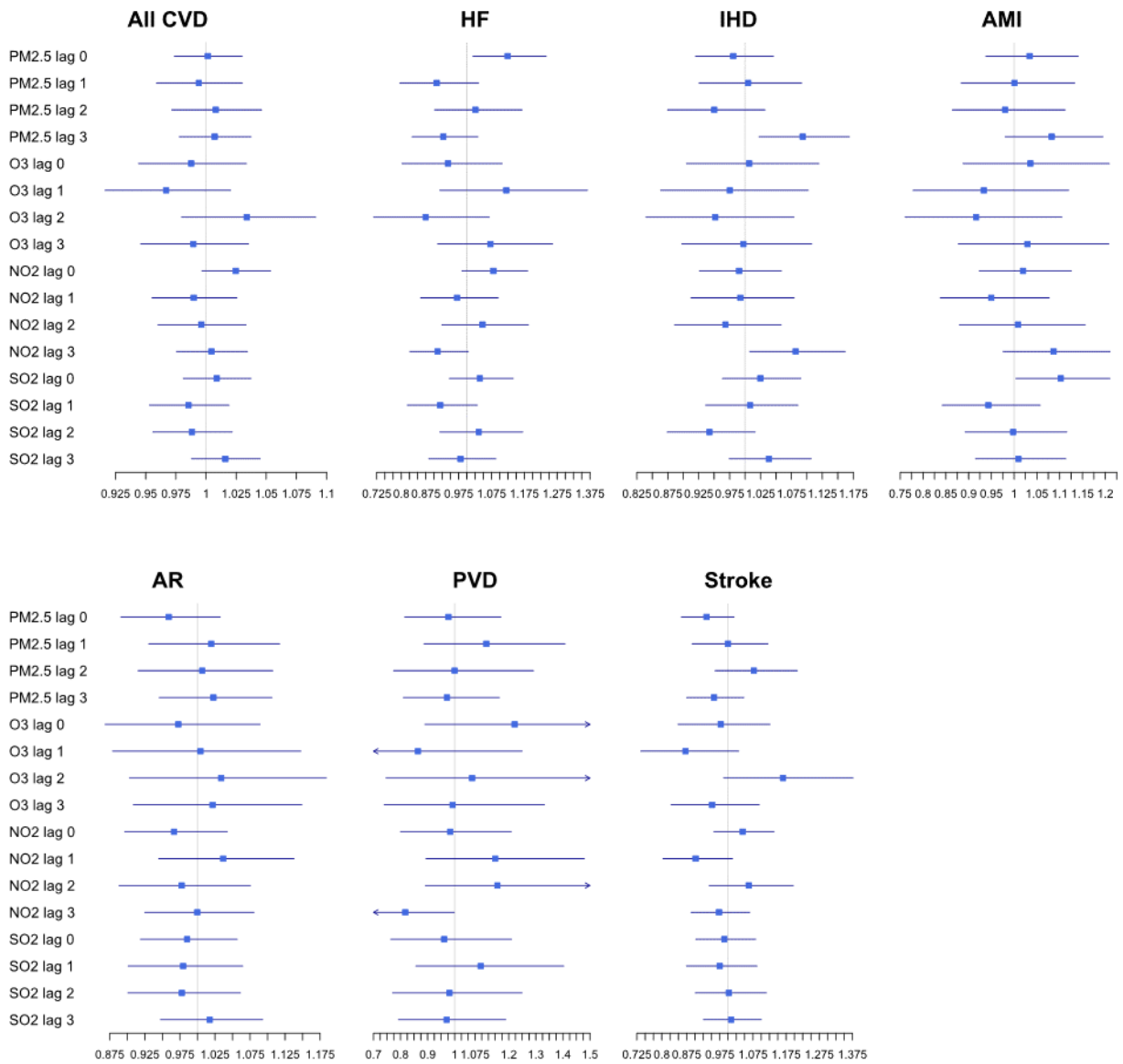

Figure S 3-8 Single Pollutant model of Criteria Pollutants and Cardiovascular Emergency Room Visits Among the People Aged 40 – 49 in Allegheny County, 1999 – 2011

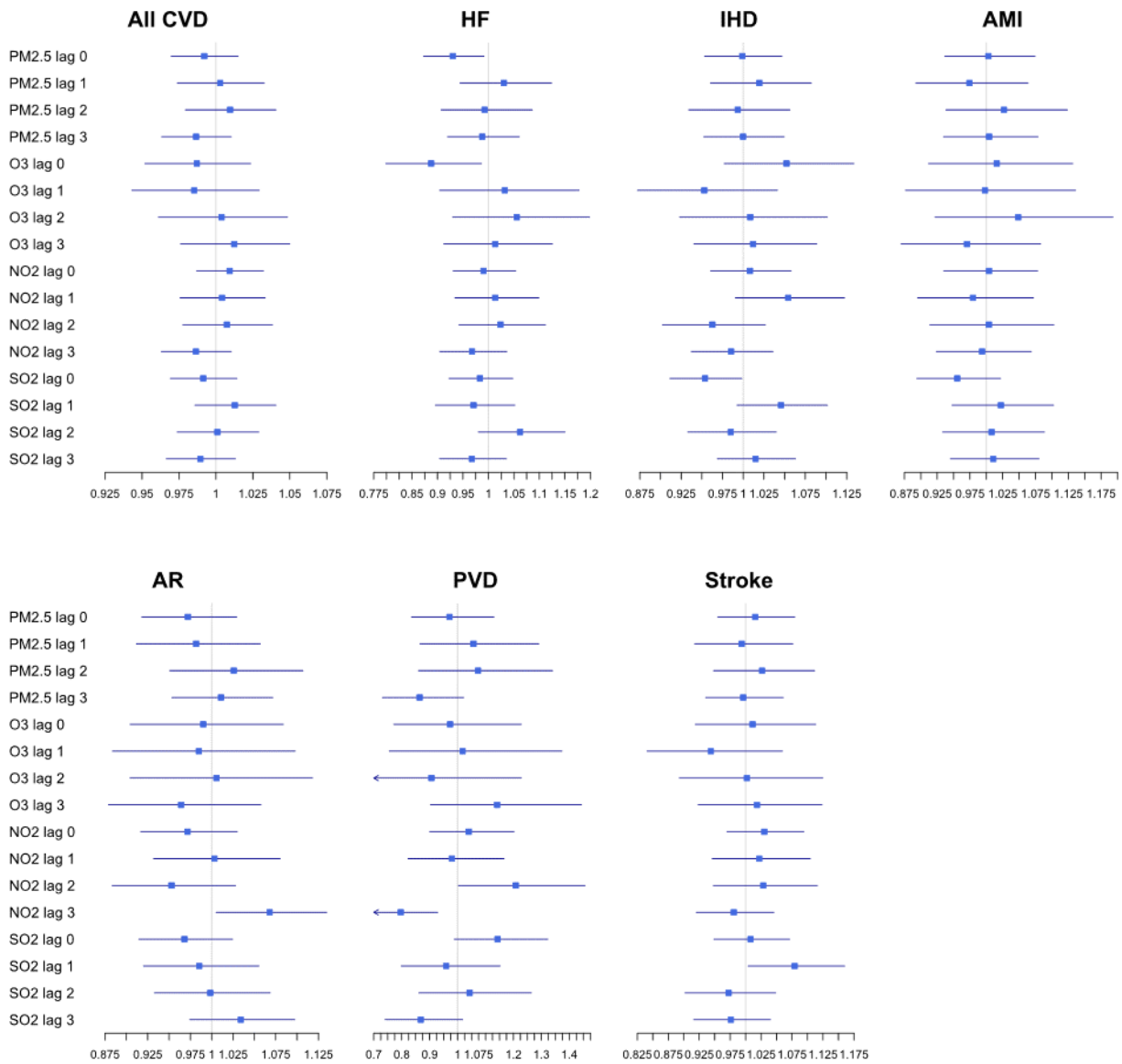

Figure S 3-9 Single Pollutant model of Criteria Pollutants and Cardiovascular Emergency Room Visits Among the People Aged 50 – 59 in Allegheny County, 1999 – 2011

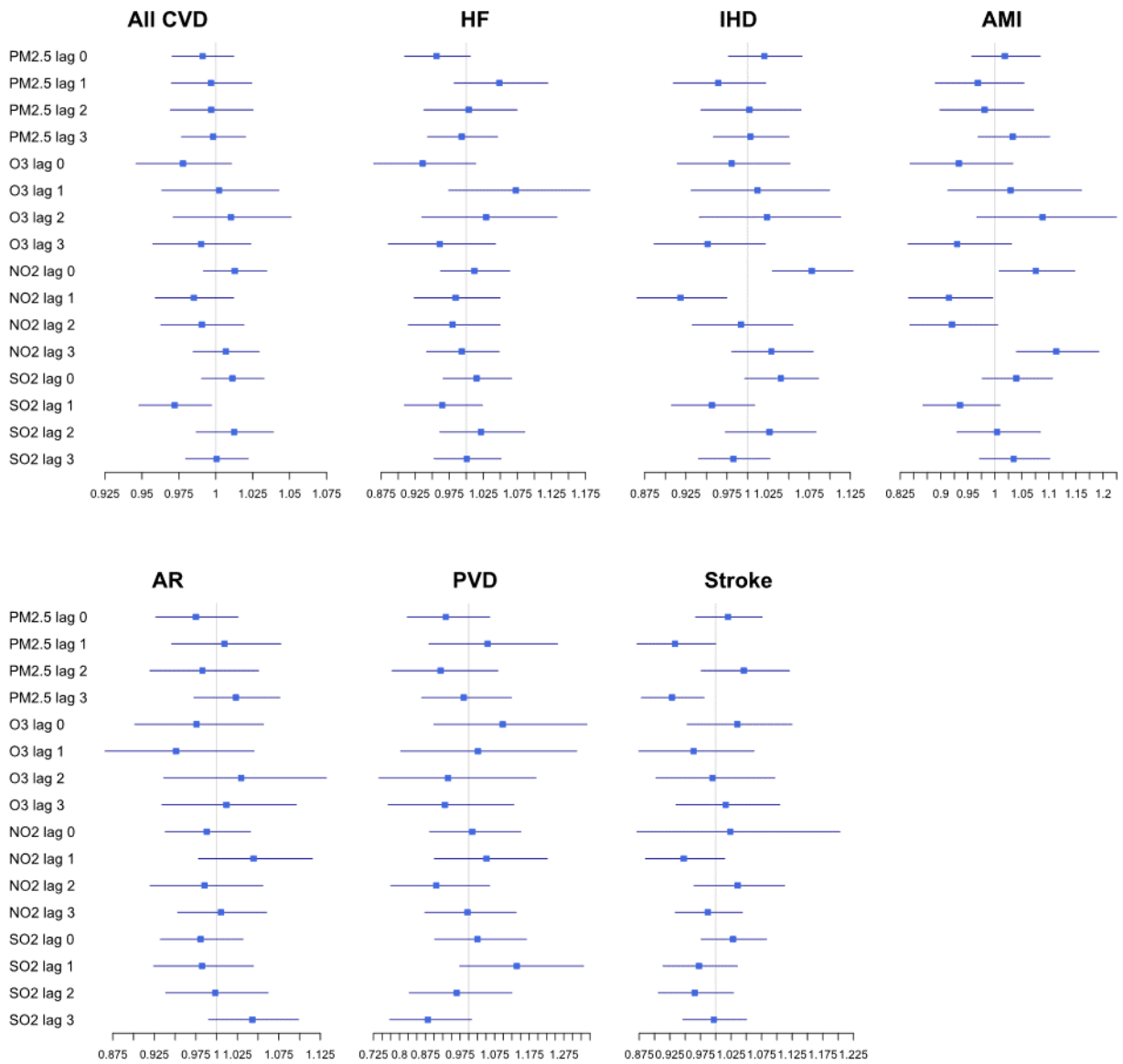

Figure S 3-10 Single Pollutant model of Criteria Pollutants and Cardiovascular Emergency Room Visits Among the People Aged 60 – 69 in Allegheny County, 1999 – 2011

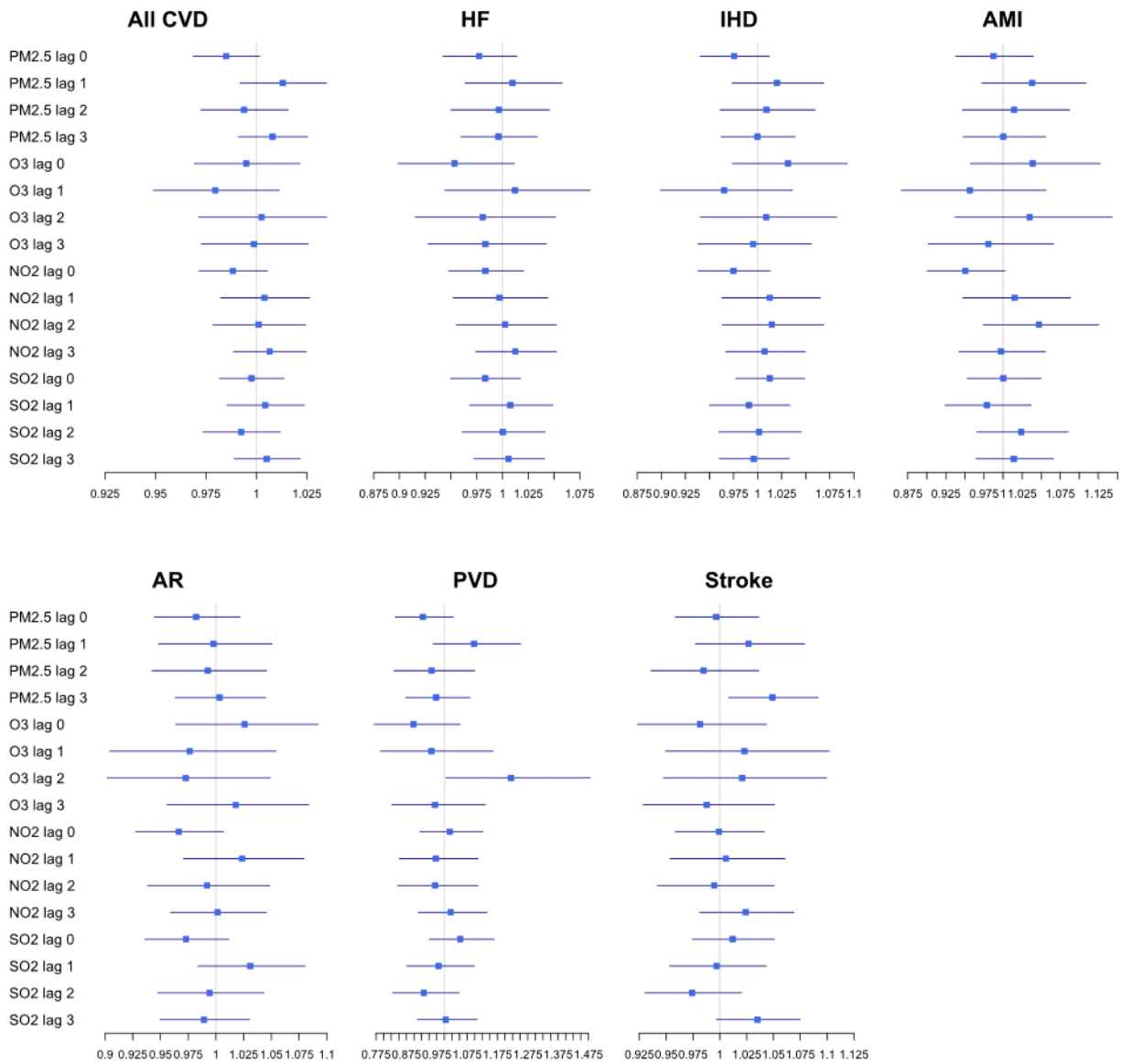

Figure S 3-11 Single Pollutant model of Criteria Pollutants and Cardiovascular Emergency Room Visits Among the People Aged 70 – 79 in Allegheny County, 1999 – 2011

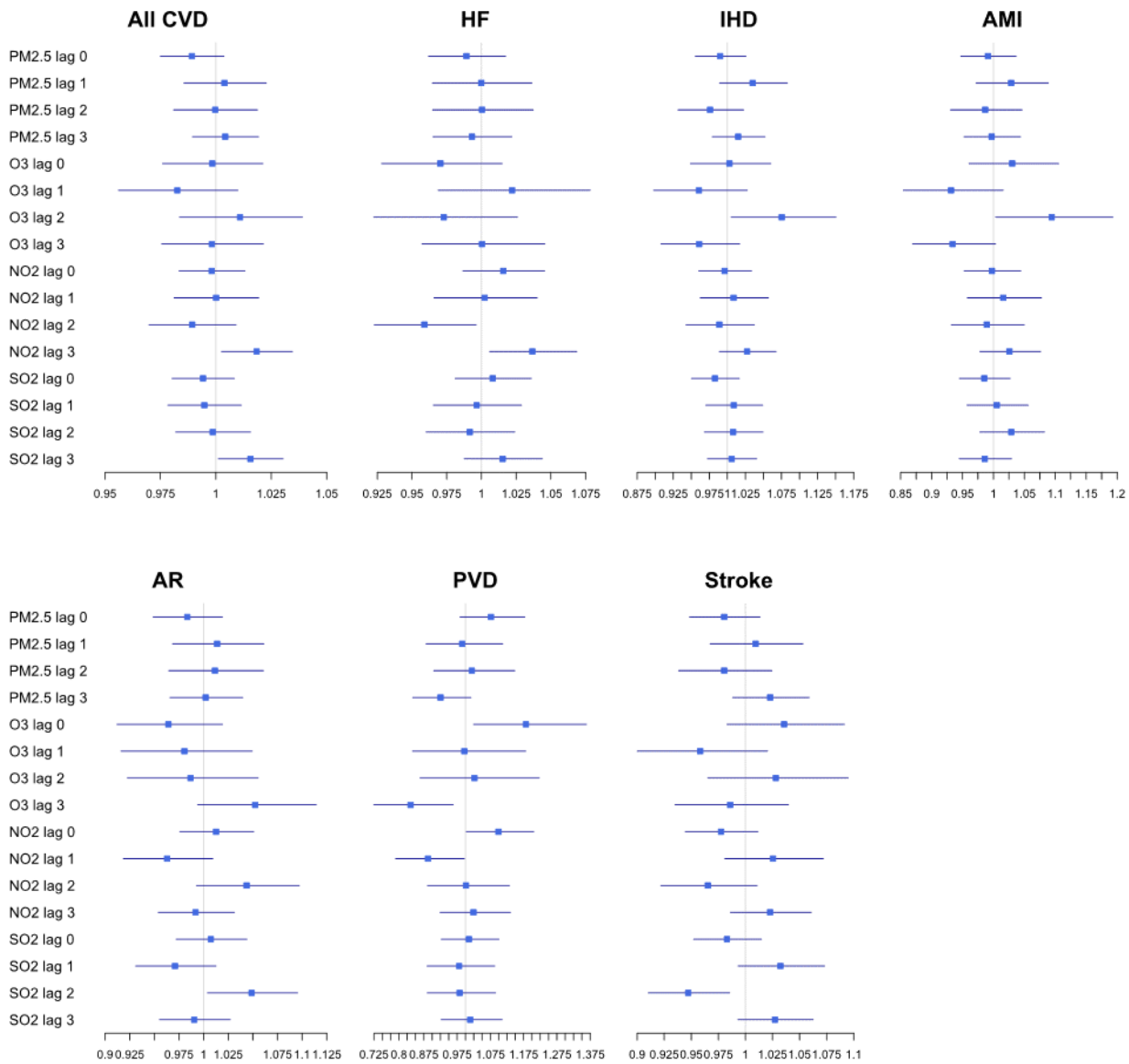

Figure S 3-12 Single Pollutant model of Criteria Pollutants and Cardiovascular Emergency Room Visits Among the People Aged 80 and older in Allegheny County, 1999 – 2011

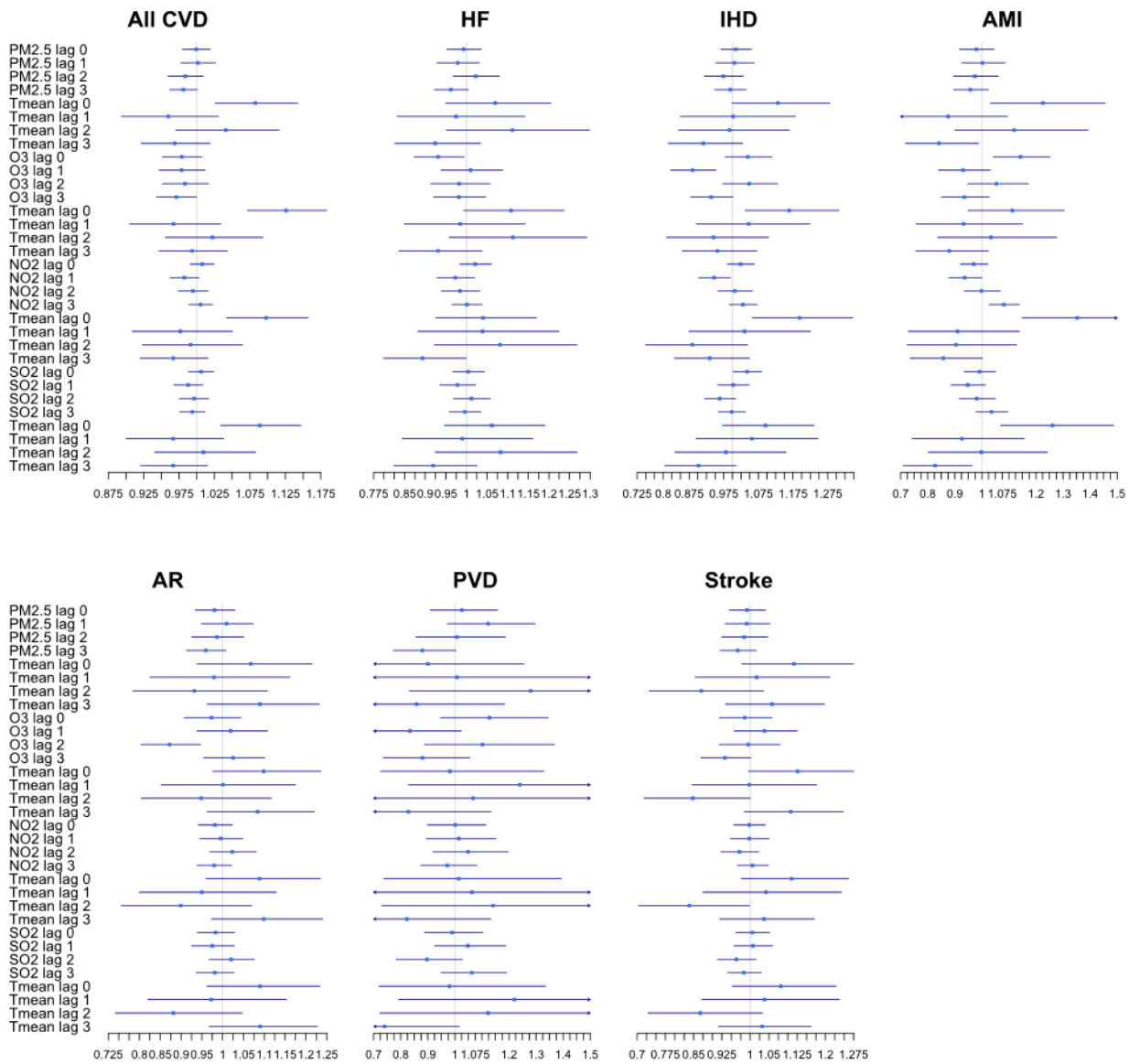

Figure S 3-13 Single Pollutant model of Criteria Pollutants and Cardiovascular Emergency Room Visits in the Spring in Allegheny County, 1999 – 2011

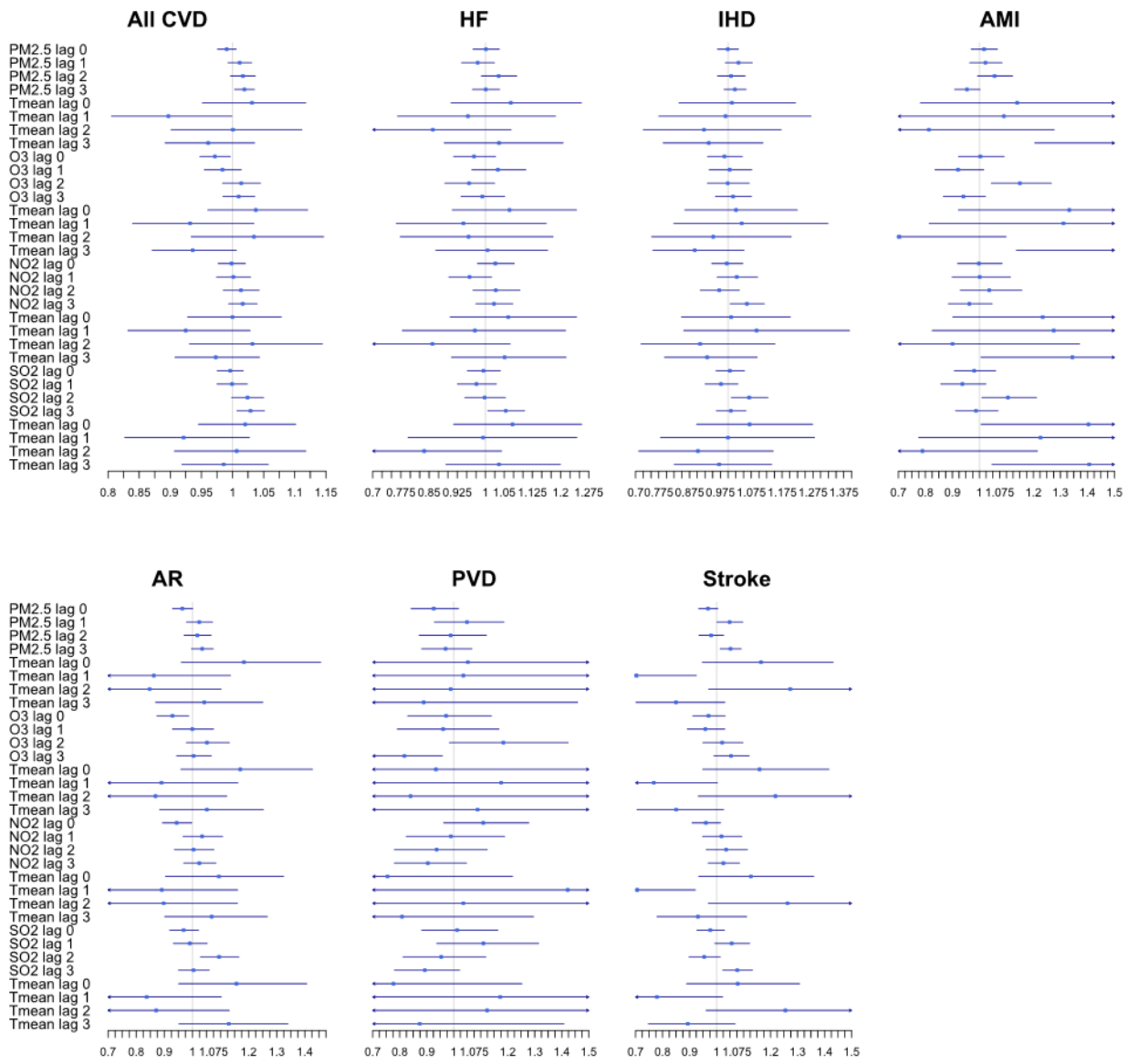

Figure S 3-14 Single Pollutant model of Criteria Pollutants and Cardiovascular Emergency Room Visits in the Summer in Allegheny County, 1999 – 2011

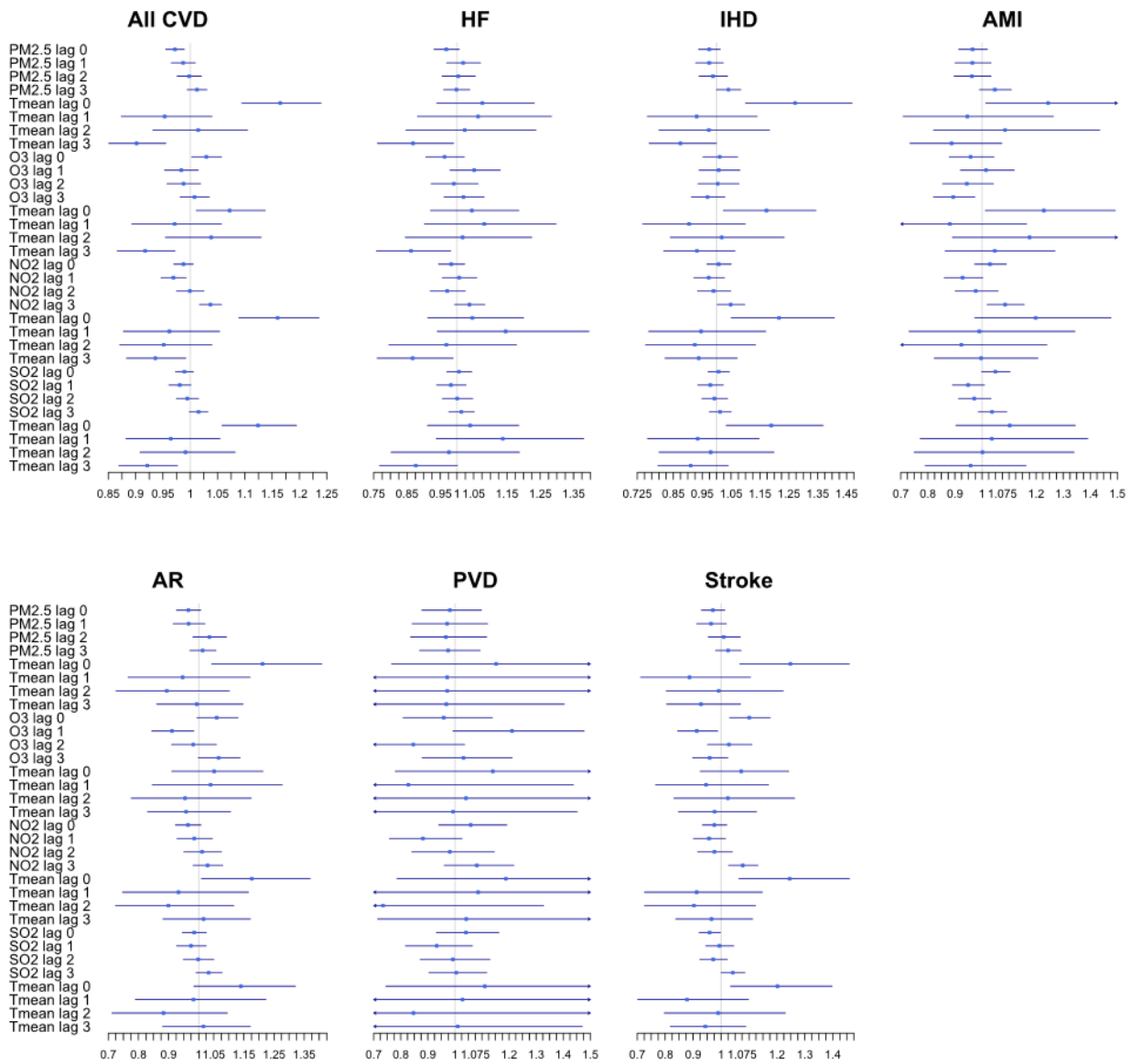

Figure S 3-15 Single Pollutant model of Criteria Pollutants and Cardiovascular Emergency Room Visits in the Fall in Allegheny County, 1999 – 2011

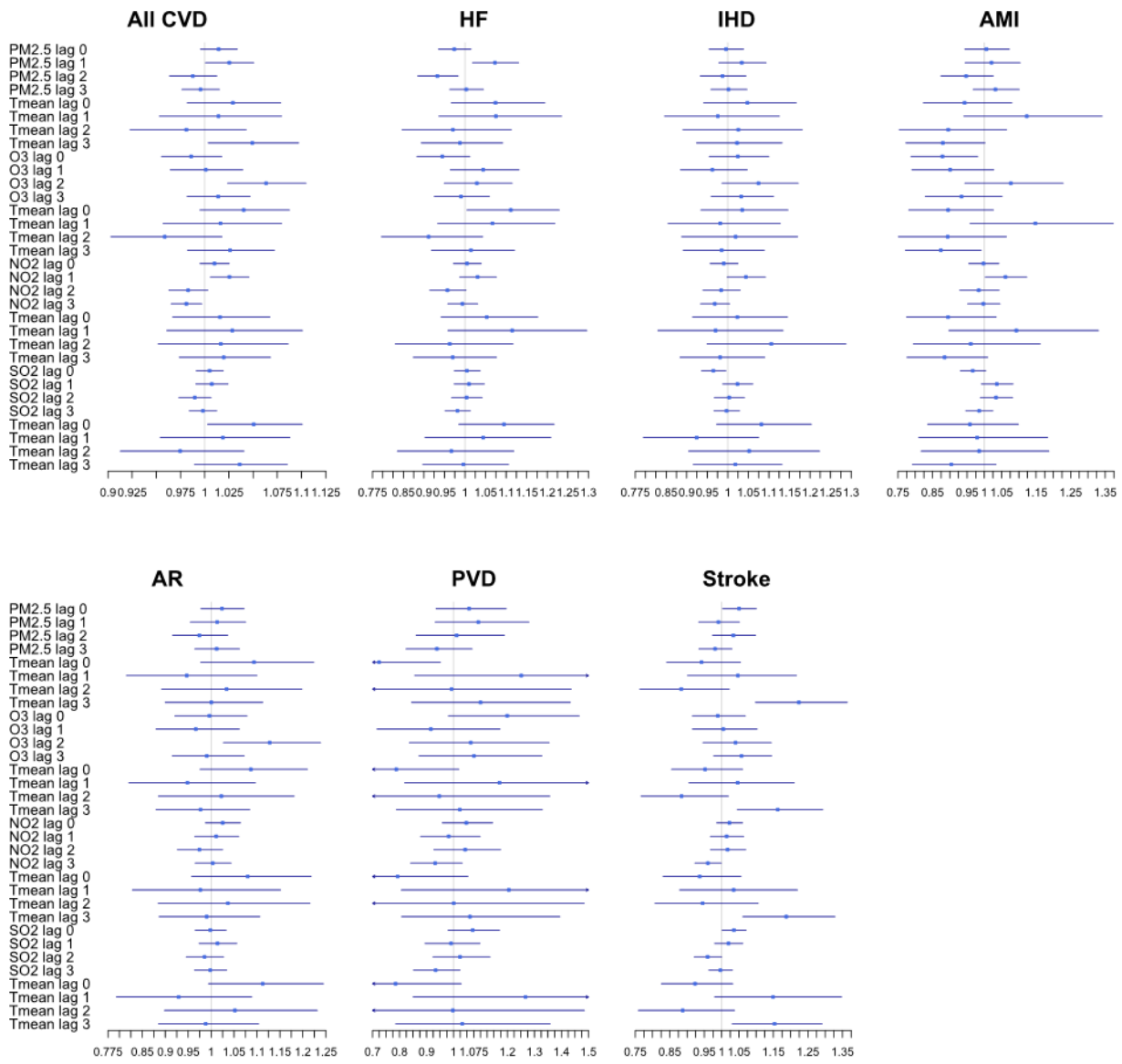

Figure S 3-16 Single Pollutant model of Criteria Pollutants and Cardiovascular Emergency Room Visits in the Winter in Allegheny County, 1999 – 2011

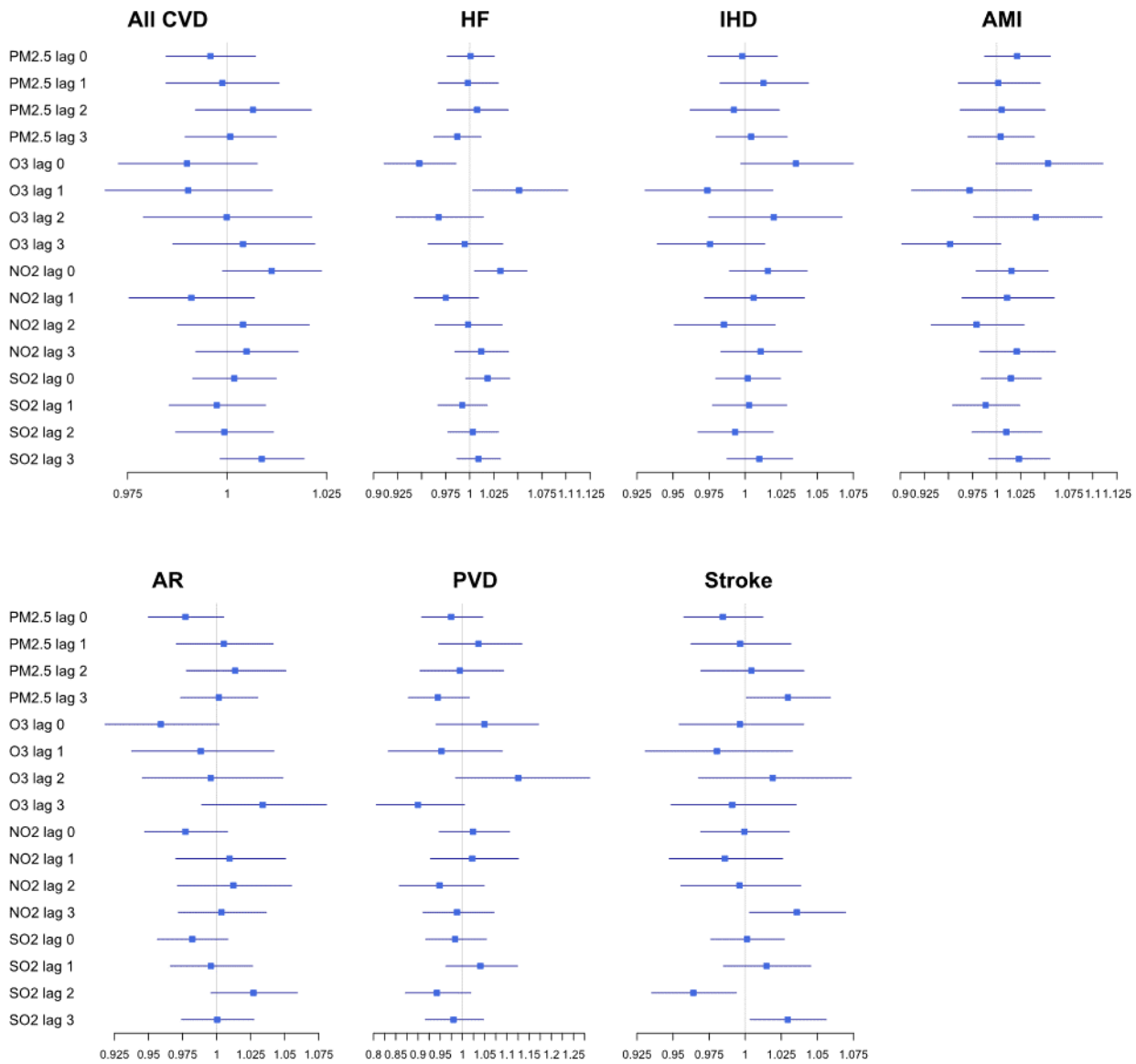

Figure S 3-17 Single Pollutant Model of Emergency Department visits of Cardiovascular Disease in Allegheny County, 1999 – 2005

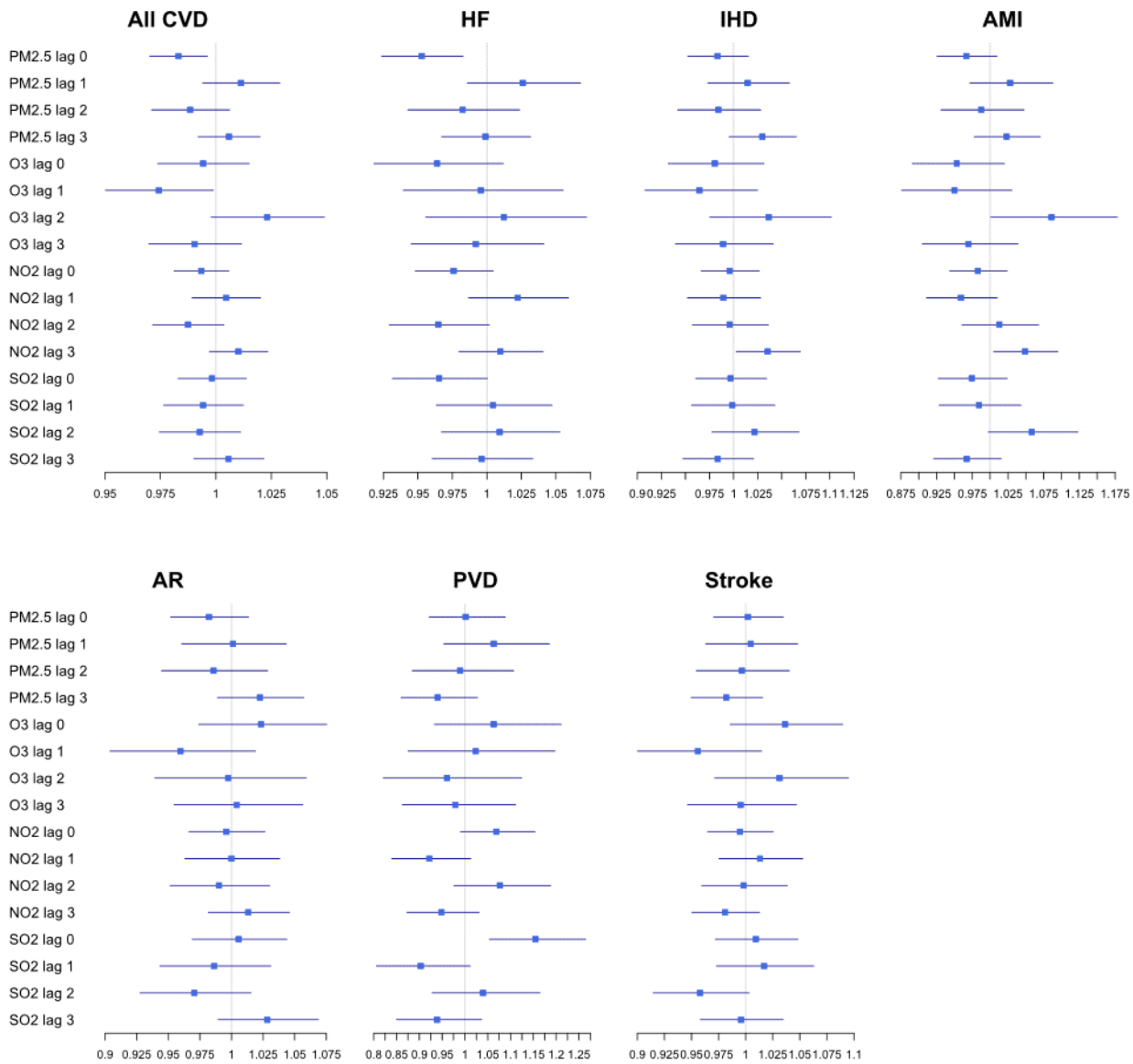

Figure S 3-18 Single Pollutant Model of Emergency Department visits of Cardiovascular Disease in Allegheny County, 2006 – 2011

4.0 LONG-TERM EXPOSURE TO $PM_{2.5}$ AND OZONE AS A PREDICTOR OF SUBCLINICAL ATHEROSCLEROSIS IN LATE MIDLIFE WOMEN: THE STUDY OF WOMEN'S HEALTH ACROSS THE NATION

4.1 ABSTRACT

Introduction: The chronic long-term exposure to air pollution and atherosclerosis is seldom studied. This paper aims to examine the association between chronic long-term exposure to $PM_{2.5}$ and ozone (O_3) and atherosclerosis using carotid intima-media thickness (CIMT) and plaque as biomarkers.

Methods: This longitudinal study was conducted among 1188 women of the Study of Women's Health Across the Nation (SWAN) from five sites with available data on both air pollutants exposure and CIMT and plaque. Yearly cumulative exposure levels of two air pollutants, particulate matter $\leq$2.5 μm ($PM_{2.5}$) and ozone (O_3), were collected from monitors 20 km within the participant's residential address at SWAN visits 3 – 7 (1999-2005). Common carotid artery intima-media thickness (CCA IMT), inter-adventitial diameter (IAD) and plaque presence was assessed at visits 12/13 (2010-2012). Linear regression models were used to estimate the effect of prior chronic long-term exposure to $PM_{2.5}$ and O_3 on mean of average and maximum CCA IMT and adventitial diameter (AD). Logistic and multinomial logistic regression was applied to assess the effects of air pollutants on plaque presence and plaque index, respectively. Full models

were adjusted for CVD risk factors, including site, age, socioeconomic status, BMI, smoking, cholesterol, triglyceride, HDL, menopause status, hormone use, fasting blood glucose, insulin, diabetic medication and hypertension, and the extended models were further adjusted for SBP and inflammatory biomarkers (tPA, PAI-1 and hsCRP).

Results: At time of carotid scan, women were on average 59.6 (±2.7) years old and a majority were postmenopausal (88.4%). The women were White (48.4%), Black (31.2%), Chinese (13.3%) and Hispanic (7.1%). Yearly mean cumulative $PM_{2.5}$ significantly predicted maximum CIMT adjusting for CVD risk factors such that 1 $\mu g/m^3$ higher cumulative exposure to $PM_{2.5}$ over 5 years was associated with an 8.0 μm (95% CI: 1.0 – 15.1) greater maximum CCA IMT at a later mid-life. This association was no longer significant after adjusting for SBP, but was not attenuated by adjusting for inflammatory biomarkers. $PM_{2.5}$ was related to mean IAD and CCA IMT when adjusting for site and socioeconomic factors, respectively, but not after adjusting for other CVD risk factors. O_3 levels were not associated with any of the outcomes. No association was found between either pollutant and plaque presence or plaque index.

Conclusions: Chronic long-term exposure to $PM_{2.5}$ was associated with mean of maximum CCA IMT later in the post-menopausal period. This association was potentially explained by SBP suggesting that early exposure to air pollutants may relate to higher values of CCA IMT potentially by increasing SBP.

Keywords: Chronic long-term exposure, $PM_{2.5}$, Ozone (O_3), Subclinical Atherosclerosis

4.2 INTRODUCTION

Air pollution is ubiquitous and has deleterious effect on health. The most significant health outcomes related to air pollution are cardiovascular (CVD) and pulmonary disease (Brook, Rajagopalan et al. 2010, Franchini and Mannucci 2012). Studies about short-term exposure to air pollution and CVD mobility and mortality have been published extensively over the past two decades (Lim, Vos et al. 2012). The long-term effect of air pollution has been less studied, and most of these studies were only considered one-year exposure of air pollutants (Atkinson, Carey et al. 2013, Green, Broadwin et al. 2016). Since air pollution exists in our everyday environment, it is possible that the cumulative effect of air pollution plays a role in heart health (Atkinson, Carey et al. 2013, Hoek, Krishnan et al. 2013).

Carotid intima-media thickness (CIMT) measured via B-mode ultrasound reflects the carotid artery wall thickness, which is a surrogate biomarker of subclinical atherosclerosis (Bots and Grobbee 2002, Bauer, Caviezel et al. 2012). CIMT reflects early vascular changes and predicts CVD and stroke events independent of traditional CVD risk factor (Chambless, Folsom et al. 2000, Bots, Evans et al. 2003, Polak, Pencina et al. 2011, Bauer, Caviezel et al. 2012, van den Oord, Sijbrands et al. 2013). CIMT has been widely used in environmental and occupational health studies to capture early atherosclerosis change (Hoffmann 2015), including studies of air pollution effects on atherosclerosis (Kunzli, Perez et al. 2011). Ultrasound assessment of plaque is a direct measure of atherosclerotic lesions. It may be a better biomarker for predicting CVD risk compared to CIMT alone (Inaba, Chen et al. 2012). Quantitative measures of plaque are an even better predictor (Naqvi and Lee 2014).

The cross-sectional relationship between exposure to one-year air pollution and atherosclerosis has been examined in several studies in different populations (Künzli, Jerrett et al. 2005, Bauer, Moebus et al. 2010, Lenters, Uiterwaal et al. 2010, Breton, Wang et al. 2012, Rivera, Basagaña et al. 2012, Tonne, Yanosky et al. 2012, Kim, Sheppard et al. 2014, Perez, Wolf et al. 2015, Su, Hwang et al. 2015). Most of these studies found that living closed to major roads and/or air pollutants were related. There are also a few published studies addressing the association between $PM_{2.5}$ and ozone (O_3) and progression of atherosclerosis (Künzli, Jerrett et al. 2010, Adar, Sheppard et al. 2013, Gan, Allen et al. 2014, Kaufman, Adar et al. 2016). However, most of these studies only have two to three years of follow-up time. Gan et al. conducted the assessment in Vancouver, Canada, where the exposure level is very low and failed to find any significant association within the whole study population. MESA Air published the 10-year follow-up results last year, and noted the $PM_{2.5}$ contributed to coronary artery calcification, but not to CIMT. Only a few studies have examined the association between very long-term exposure to air pollution and atherosclerosis, and two of these studies were limited to very young populations, whose atherosclerotic changes were not profound. Rivera et al. estimated a 10 year exposure of NO_2 using land use regression and traffic proximity related to CIMT and ankle-branchial index (ABI) among the community population aged 32 – 86 year-old in Girona region in Spain (the REGICOR study) (Rivera, Basagaña et al. 2012). NO_2 and traffic proximity was related to CIMT in the unadjusted model, but only traffic proximity remained significantly associated with CIMT in the model adjusted for confounders. With extremely limited studies between long-term air pollution exposure and subclinical atherosclerosis, more investigations are needed to establish this association.

We, therefore, investigated the association between five-year exposure to $PM_{2.5}$ and O_3 and atherosclerosis burden in about 6.6 (range: 5.4 – 9.6) years later, utilizing both CIMT and plaque as biomarkers of atherosclerosis as outcomes in a cohort of women as they transition through the menopause. We hypothesized that women who are exposed to higher levels of $PM_{2.5}$ and O_3 over time will have a higher value of CIMT or plaque index.

4.3 METHODS

4.3.1 Study Population

The Study of Women's Health Across the Nation (SWAN) is a community-based multi-center multi-ethnic cohort study to observe women's health transitioning through menopause. The SWAN study, conducted at seven sites (Boston, MA; Detroit, MI; Oakland, CA; Los Angeles, CA; Pittsburgh, PA; Chicago, IL; and Newark, NJ) across the U.S. began in 1996. There were 3302 women aged 42 – 52 years enrolled at baseline. Each site recruited White and another minority racial group. Oakland, CA recruited Chinese women; Los Angeles, CA recruited Japanese women; Newark, NJ recruited Hispanic women; and the other four sites recruited Black women. Participants were invited back for a follow-up visit after one or two years. The study was approved by the Institutional Review Board at each site. Written informed consents were obtained from all of the participants (Sowers, Crawford et al. 2000).

4.3.2 Exposure to $PM_{2.5}$ and O_3

Air pollution exposure was assessed by the Air Pollution Study, an ancillary study of SWAN. This study determined the air pollutant exposure levels of SWAN participants based on the residential address at six sites, not including Boston, MA site. Daily $PM_{2.5}$ and O_3 values were retrieved from US EPA Air Quality System DataMart (US EPA 2015). Exposure to $PM_{2.5}$ and O_3 was determined by monitors within 20 km from each participant's address. Annual exposure to these pollutants was defined as exposed 360 days before the study visit. Detailed methods of exposure assessment have been published elsewhere (Ostro, Malig et al. 2014, Green, Broadwin et al. 2016). These data were collected for a one-year exposure period prior to each of the SWAN visits 3 – 7, when most of these women were at menopausal transitional status. At visits 5, 5.0% were pre-, 43.0% were early peri-, 11.6% were late peri, and 40.3% were post menopause, among the women who we can determine their menopausal status. An annualized mean cumulative level of air pollutants was calculated based on the yearly exposure prior to the SWAN visits 3 – 7. Area under the curve was calculated by the trapezoidal rule and divided by the year of exposure estimation.

4.3.3 Assessment of CIMT and plaque

Ultrasound scans were implemented at SWAN visits 12 or 13 at six participant sites, excluding Los Angeles, CA site. At each site, centrally trained and certified sonographers obtained carotid ultrasound images using a Terason t3000 Ultrasound System (Teratech Corp, Burlington, MA). Participants were scanned in the supine position with the head turned 45° toward the side opposite the side being examined. The carotid artery was imaged in its long axis with multiple

scanning angles (anterior, lateral, and posterior) and the angle of interrogation resulting in the thickest IMT was used for later reading. Two images were obtained of each of the left and right distal common carotid artery (CCA) for later reading. The inter-sonographer reliability for repeat carotid scan was good to excellent, with intercorrelation coefficients (ICC) for mean CCA IMT 0.72 – 0.95 and for IAD 0.80 – 0.98. Carotid scan images were read centrally at the SWAN Ultrasound Reading Center (University of Pittsburgh Ultrasound Research Lab). The reading reproducibility was excellent between and within readers, with ICC greater than 0.87. AMS semi-automated edge detection software were used to semi-automatedly measure the near and far wall CCA intima-media thickness (IMT) by tracing the lumen-intima interface and the media-adventitia interface across a 1-cm segment proximal to the carotid bulb (Wendelhag, Gustavsson et al. 1991). The average and maximal values for these measures were recorded. The mean of the average and maximal CCA IMT readings of all 4 images were applied in analyses. CCA inter-adventitial diameters (IAD) were measured directly as the distance from the adventitial-medial interface on the near wall to the medial-adventitial interface on the far wall at end-diastole across the same CCA segments used for IMT measurement. Sonographers at each site evaluated the presence and extent of plaque in each of 5 segments of the left and right carotid artery (distal and proximal CCA, carotid bulb, and proximal internal and external carotid arteries). Plaque was defined as a distinct area protruding into the vessel lumen that was at least 50% thicker than the adjacent IMT and summarized as the presence or absence of any plaque. Additionally, for each segment, the degree of plaque was graded between 0 (no observable plaque) to 3 (plaque covering 50% or more of the vessel diameter). The grades from all segments of the combined left and right carotid artery were summed to create the plaque index, a measure of plaque severity (Thompson, Sutton-Tyrrell et al. 2001, Sutton-Tyrrell, Kuller et al. 2002).

4.3.4 Assessment of other CVD risk factors

Covariates were extracted from SWAN baseline or SWAN follow-up visits. A detailed illustration of when the data were collected, and how the exposures, outcomes and co-variances were extracted are presented in Table 4-1. Self-reported race, and education were collected at the baseline screening visit. Financial strain was collected based on the question of how hard to pay for the very basics, which includes living expenses and medical treatment. And, it was categorized into three classes: very hard, somewhat hard, and not hard at all. This variable was collected at baseline, and visits 6 to 13. A longitudinal category for financial strain was created and categorized into three groups, (a) consistently not hard, if the participants had no difficulties to pay for the basics at all at all visits; (b) mixed, if the participants had more than 50% of time had no difficulties; (c) consistently hard, if the participants had more than 50% of time had any difficulties (Thurston, El Khoudary et al. 2014). Smoking, diabetes (DM), hypertension (HTN), and medication use of DM and HTN, menopausal status and hormone use were asked at baseline, as well as each of follow-up visits. The statuses of these categorical variables at the time of carotid visits were determined based on this longitudinal data collection, e.g. DM (Yes/No), HTN (Yes/No) ever take the medications (Yes/No). Smoking was categorized as never smokers, past smokers, and current smokers. Physical measures of body mass index (BMI), and systolic and diastolic blood pressures (SBP and DBP) were collected at all the visits using the standard methods. We extracted the data of visits 3 – 7 incorporating with the air pollution exposure measures. Blood samples were draw after a 12-hour fasting. The samples were sent to the Medical Research Laboratories for analysis. The analyses of high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), total cholesterol, triglyceride, as well as high sensitivity C-reactive protein (hsCRP), tissue-type plasminogen activator antigen

(tPA), and plasma plasminogen activator inhibitor 1 (PAI-1) used the standard methodology, and has been published elsewhere (El Khoudary, Wang et al. 2016). However, the blood sample data were not collected at SWAN visits 8 – 11. As we only have the air pollution estimation available at SWAN visits 3 – 7, a cumulative mean level of all these continuous variables was calculated using corresponding SWAN visits 3 – 7 data.

Table 4-1 Timeline of the data collection and extraction of this analysis

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<tr><td>00</td><td>01</td><td>02</td><td>03</td><td>04</td><td>05</td><td>06</td><td>07</td><td>08</td><td>09</td><td>10</td><td>11</td><td>12</td><td>13</td></tr>
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<tr><td></td><td></td><td></td><td colspan="5">Air Pollution Exposure assessment: $PM_{2.5}$ and ozone</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td></td><td colspan="5">Other continuous co-variates*</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Race, SES</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
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</table>

* BMI, SBP, cholesterol, triglyceride, LDL-c, HDL-c, fasting blood glucose, insulin, hsCRP, tPA, and PAI-1

4.3.5 Statistical analysis

This analysis was based on data from the five sites that had both exposures and outcomes data available: Detroit, MI; Oakland, CA; Pittsburgh, PA; Chicago, IL; and Newark, NJ. Due to their non-normal distribution, glucose, insulin, triglyceride, hsCRP, tPA and PAI-1 were log transformed. Unadjusted associations between $PM_{2.5}$ quartiles and covariates and outcomes were examined by linear regression or logistic regression. Linear regression was used to estimate the effect of visits 3 – 7 annualized mean exposure level of $PM_{2.5}$ and O_3 and mean of average and

maximum CCA IMT, and IAD at visits 12/13. Logistic regression was used to evaluate the relationship between the annualized mean exposure of air pollutants and plaque presence. Multinomial logistic regression was used to determine the association between the annualized mean exposure of air pollutants and plaque index. Plaque index was categorized into three groups, plaque index as 0, which were also with no plaque, plaque index as 1 – 2, and plaque index greater than 2. Three models were constructed to establish the association between air pollutant exposures and subclinical atherosclerosis, (1) unadjusted model, controlling for site, (2a) model adjusted for SES/demographic factors, age, race, education, and financial strain, (2b) fully adjusted model, further adjusted for traditional CVD risk factors, BMI, smoking, triglyceride, cholesterol, HDL-c, hypertension mediation, menopause status, hormone replacement therapy, fasting glucose, and diabetes medication and (3) extended model, additionally adjusted for potential mediators, SBP, hsCRP, tPA and PAI-1. The main analyses are single pollutant models, which used $PM_{2.5}$ or O_3 as the exposure. A two-pollutant model was also fitted for the supplementary analyses, which included both $PM_{2.5}$ and O_3 in the same model. Analyses were performed with SAS (V9.3, SAS Institute, Cary, NC). All tests were 2-sided, $\alpha = 0.05$.

4.4 RESULTS

This study included 1188 women who participated in the SWAN study, who had both air pollutant estimates at visits 3 – 7 and who had carotid scan at visits 12/13. We excluded participants who had an MI and stroke before the carotid ultrasound scan. There were 573 White, 373 Black, 159 Chinese, and 83 Hispanic women with a mean age of 59.6 years (SD = 2.7,

range: 54 – 67), and about 90% of them were post-menopausal. The annualized mean $PM_{2.5}$ exposure level was 14.9 (SD = 1.9) $\mu g/m^3$ (IQR: 13.6 – 16.1) for all the five study sites; and the annualized mean O_3 exposure level was 30.8 (SD = 6.2) ppb (IQR: 29.2 – 35.9). The within site exposure level of these two pollutants at baseline and follow-up are shown in Figure 3-1. Oakland, CA had the lowest $PM_{2.5}$ exposure over time, with a mean of 12 $\mu g/m^3$ (SD = 0.6), and relative low level of O_3 exposure, but the range was very wide. All other four sites had comparable $PM_{2.5}$ exposure levels. Detroit, MI site was excluded for O_3 exposure, since only four participants had yearly O_3 exposure data available. The mean of the average CCA IMT was 0.792 μm (SD = 0.120), the mean of maximum CCA IMT was 0.927 μm (SD = 0.140) and the mean of the average IAD was 7.200 μm (SD = 0.667). The correlation between the two pollutants $PM_{2.5}$ and O_3 was about 0.56. The correlation between the main outcomes of mean of average and maximum CIMT was 0.96, and the correlations between these two measures and IAD were moderate about 0.57. The correlation between the exposures and outcomes were very low (Table 4-3). Baseline characteristics and cumulative CVD risk factors by annualized cumulative mean $PM_{2.5}$ quartiles are presented in Table 4-2. Most of these factors had a positive relationship with $PM_{2.5}$. Black and Hispanic were more likely to live in the areas with higher $PM_{2.5}$ exposures, while all Chinese women lived in area within the lowest quartile of $PM_{2.5}$ exposures. We must keep in mind that each site of SWAN only recruited another minority group, thus this reflected the differences in $PM_{2.5}$ exposures by sites. Never smokers, and women who reported no financial strains were more likely to reside in lower $PM_{2.5}$ exposure areas. Women who lived in more polluted areas were more likely to have a higher BMI, BP, LDL-c, insulin, hsCRP, tPA and PAI, and lower HDL. They also were more likely to be hypertensive, diabetic and to take medications. We also observed that women in the higher $PM_{2.5}$ exposure quartile had

higher values of mean of average and maximum CCA IMT; but was less likely to have plaque. In our population, Chinese women were more likely to have plaque, this may be the reason driven the higher percentage of plaque in this lower $PM_{2.5}$ exposure quartile.

In the main analyses of CCA IMT measures, $PM_{2.5}$ was associated with mean of the average CIMT in the model adjusted for site and SES (Table 4-4). Women who were exposed to 1 $\mu g/m^3$ higher $PM_{2.5}$ had a 7.57 μm (95% CI: 1.31 – 13.82) thicker CIMT. However, this association was only marginally significant in the fully adjusted model: per 1 $\mu g/m^3$ higher exposure to $PM_{2.5}$ was related to 5.59 μm (95% CI: -0.58 – 11.75) thicker CIMT. The linkage between $PM_{2.5}$ and mean of the maximum CIMT was statistically significant in the fully adjusted model. Women with an average of 1 $\mu g/m^3$ higher exposure to $PM_{2.5}$ during a 5-year period, had an 8.03 μm (95% CI: 1.01 – 15.05) thicker mean of the maximum CIMT on average (Table 4-5). After further adjusting for potential mediators, this association became null, which was majorly influenced by systolic blood pressure. The association between $PM_{2.5}$ and IAD was only revealed in the unadjusted model, but not in the fully adjusted model (Table 4-6). No clear association was observed between O_3 and any of CIMT measures (Table 4-4, Table 4-5 and Table 4-6). There was no clear association between these two pollutants and plaque presence or plaque index (Table 4-7 and Table 4-8).

When stratifying by characteristics and CVD risk factors, we observed that $PM_{2.5}$ had a higher impact on the mean of the maximum CCA IMT values in some of the subgroups (Figure 4-2). However, due to the disproportional sample sizes among the strata, no interaction was found. The relationships were stronger among the diabetic, obese and more than college educated women.

4.5 DISCUSSIONS

In this long-term observational study, we found that women who had a higher cumulative exposure to $PM_{2.5}$ over approximately 5 years during early midlife had a higher mean of the maximum CCA IMT at post-menopause, controlling for traditional CVD risk factors. Associations with $PM_{2.5}$ and mean of the average CCA IMT and IAD was in the minimal model, but not independent from the CVD risk factors. However, no association was found with plaque and O_3.

This is the first study focused on the effects of long-term air pollution on atherosclerosis among women transitioning from peri-menopause to post-menopause. There were very limited studies focused on the very long-term exposure. Besides our study, there is only one study in a middle to old aged community population, who were at higher risk of atherosclerosis (Rivera, Basagaña et al. 2012). What is unique to our study is that women are at an accelerated CVD risk during this period of their life. An earlier analysis utilizing SWAN Pittsburgh cohort indicated that late peri-menopausal women are at the increased risk of CVD, and have the fastest progression of CIMT (El Khoudary, Wildman et al. 2013). During the time of the exposure, most of these women were at a menopausal transitioning stage. Thus, exposure data collected at this life stage may also have an impact on their atherosclerosis and CVD risk.

In this study, we revealed that women who were exposed to 1 $\mu g/m^3$ higher $PM_{2.5}$ had an 8.03 μm (95% CI: 1.01 – 15.05) thicker mean of the maximum CCA IMT, adjusting for traditional CVD risk factors. Rivera and colleagues found that traffic intensity was associated with CCA IMT, and CIMT at six segments, which also including ICA and BULB segments (Rivera, Basagaña et al. 2012). Traffic is one of the major sources of $PM_{2.5}$ in the urban area (U.S. Environmental Protection Agency 2014), and it also can emit other pollutants, e.g. NO_2,

and produce noises. It is a measure of near-road pollution level, but not necessary the "true" value of any pollutants. This may reflect that the association between multi-pollutants from a single source, traffic, and CIMT is much stronger. However, we did not see any association in our two-pollutant model when put both pollutants in the same model (supplementary tables). Accounting for traffic alone cannot reflect the other sources of air pollutants, like factories in the region. They found that NO_2 was related to six-segment measure of CIMT in the unadjusted model, not in the fully adjusted model. As, these two pollutants were moderately correlated, it is not an ideal method to account for the multi-pollutant effect. There was a multi-collinearity issue, such that the standard deviation of the coefficient of $PM_{2.5}$ was inflated about 36.4%. Also, in our study, the two-pollutant model was not comparable to the single pollutant model with $PM_{2.5}$ as there was a significant sample size differences between the two analyses due to extensive missing values of O_3 exposure in Detroit, MI site.

Although there are limited long-term exposure to air pollutants and sub-clinical atherosclerosis studies, the relationship between $PM_{2.5}$ and CIMT has been observed in several cross-sectional and longitudinal studies (Bauer, Moebus et al. 2010, Künzli, Jerrett et al. 2010, Adar, Sheppard et al. 2013, Sun, Kaufman et al. 2013, Kim, Sheppard et al. 2014, Su, Hwang et al. 2015, Kaufman, Adar et al. 2016). The effect of air pollution on CVD has been extensively reviewed in several papers (Brook 2008, Brook, Rajagopalan et al. 2010, Franklin, Brook et al. 2015), and all of them concluded the air pollution contributes about 15% – 30% of CVD in the proportion explained by the traditional risk factors. There are three possible pathways and two of them have a chronic effect on CVD. $PM_{2.5}$, given its small size, can penetrate deeply into alveoli, and get into the body system. It can have a direct effect on the endothelial layer of the blood vessel, which can cause endothelial dysfunction, mediating reactive oxygen species (ROS) to add

oxidative stress, and increase blood pressure, and possibly lead to atherosclerosis (Langrish, Bosson et al. 2012). Another mechanism is that PM can also trigger systemic oxidative stress and inflammation, increase adipokines and cytokines expression level, oxidize lipids, inflamed fat cells and thus, lead to atherosclerosis. However, in our analyses, we did not see an effect size change by adding hsCRP, tPA and PAI-1 in the fully adjusted model, which is consistent with findings from MESA Air study (Adar, Sheppard et al. 2013, Kaufman, Adar et al. 2016). We found that elevated systolic blood pressure and hypertension may be a factor mediating the effect of $PM_{2.5}$ on CIMT, by reducing the effect size by about 37%. There is ample evidence for the effects of $PM_{2.5}$ on hypertension and elevation of blood pressure (Franklin, Brook et al. 2015), risk factors for atherosclerosis and CVD events (Allen, Siddique et al. 2014). It is plausible that elevated blood pressure can be a mediator of the effect of $PM_{2.5}$ on atherosclerosis.

In our stratification analyses we showed that obese and diabetic women were more susceptible to $PM_{2.5}$, as with each 1 $\mu g/m^3$ higher exposure to $PM_{2.5}$, they had a much thicker mean of maximum CCA IMT compared to their counterparts. We did no treat these factors as potential intermediates, but the effect size of the fully adjusted model decreased about 20% compared to model adjusted for socioeconomic status. In the paper published by Rivera et al, they treated these factors as intermediated factors (Rivera, Basagaña et al. 2012). Besides the potential mechanism of $PM_{2.5}$ and atherosclerosis by blood pressure, there are potential other pathways of $PM_{2.5}$ lead to atherosclerosis. In our stratification analyses, we revealed that obese and diabetic women had a higher effect size of the relationship of $PM_{2.5}$ and maximum CCA IMT than their counterpart. Also, in several animal studies they used the APOE or LDLR knock-out mice showed an increase in plaque volume by 50% after exposing to $PM_{2.5}$ (Sun, Wang et al. 2005, Araujo, Barajas et al. 2008, Soares, Carvalho-Oliveira et al. 2009). This may indicate that

$PM_{2.5}$ increase the mice's susceptibility to fat, and therefore, lead to atherosclerosis. The association between $PM_{2.5}$ exposure and diabetes has been demonstrated in several newly published papers (Eze, Schaffner et al. 2014, Park, Adar et al. 2015, Weinmayr, Hennig et al. 2015). Diabetes may also interplay with oxidative stress and increase the risk in developing atherosclerosis (Beckman, Creager et al. 2002, Bullon, Newman et al. 2014). In the Nurses' Health Study, they also observed the women with diabetes who exposed to higher level of $PM_{2.5}$ had an increased risk of incidence CVD, coronary heart disease and stroke (Hart, Puett et al. 2015). Although we cannot determine which pathway related to $PM_{2.5}$ inducing atherosclerosis, it seems that women with diabetes and obesity are more susceptible to $PM_{2.5}$ exposure.

In this analysis, we did not find evidence of an association between O_3 exposure and any biomarkers of subclinical atherosclerosis. There are very few studies examining the long-term exposure effect of O_3, since ambient O_3 level is largely dependent on the ultra-violate intensity, which is much higher during the warm season, and much lower during the cold season (U.S. Environmental Protection Agency 2015). And, the acute effect of O_3 can be reversible by the healthy human system (Allen 2002). So, the long-term effect of O_3 is very difficult to observe in the areas with higher level during the summer, and very low levels during the winter. In our sample, the annualized mean level of the O_3 exposure in our study was about 30.8 (range: 17.8 – 46.1) ppb, which was much lower than the national standard of 70 ppb (U.S. Environmental Protection Agency 2017). The only study examining O_3 exposure was among the college student in Los Angeles, CA (Breton, Wang et al. 2012). They noted that the early childhood exposure in their 6 – 12-year-old had an impact on their CIMT at age 19 years, and not the exposure period right before the ultrasound scan. Most of the participants in this study were originally from southern California, which may indicate that they were exposed to high levels of O_3 all year

round. Although Los Angeles, CA is one of the SWAN site, we did not collect CIMT measures among these women; therefore, we do not have compatible data to compare to this study.

We did not observe any association between either pollutant with plaque presence or plaque index. So far, there have been only one published study using plaque as an outcome (Gan, Allen et al. 2014). Consistent with our findings, they did not find much association between any of the pollutants, e.g. traffic, $PM_{2.5}$, NOx, and noise, with plaque in the overall population. However, they observed that among Chinese population, living close to major roads was associated with bigger plaque area. As we discussed earlier, traffic may not reflect the true value of the exposure to certain pollutants. There is very weak evidence between $PM_{2.5}$ and plaque in the current literature. We did not find any association between either pollutants and plaque outcomes among Chinese. However, as Chinese women only were recruited in the Oakland, CA site, where pollutant levels were the lowest among all the sites, it is hard for us to observe any significant association among them. Furthermore, our study population may be still young to observe plaque. Although there are about 45% women who had plaque, most of them had only one or a few small plaques with plaque index of 1 – 2. The subclinical phase of atherosclerosis is very long (Künzli, Perez et al. 2011, Juhola, Magnussen et al. 2013). Thus, at this stage, a plaque assessment may not yet reflect extended subclinical atherosclerosis among these women. Especially for the women who at a very early stage, those women may develop carotid wall thickening, but not yet plaque. CIMT, on the other hand, can reflect arterial wall thickening as well as possible plaques. Therefore, it is a widely-used measure for the studies among women in this age group, and in environmental studies (Bots and Grobbee 2002, Bauer, Caviezel et al. 2012, Brucker, Moro et al. 2013).

When stratified by the traditional CVD risk factors, we noted that some subgroups were more susceptible to residential $PM_{2.5}$ exposure than their counterparts. Surprisingly, women with post-college education had a higher response to $PM_{2.5}$ exposure is unexpected. However, all the other studies categorized education as lower or higher than high school (Adar, Sheppard et al. 2013, Gan, Allen et al. 2014, Kaufman, Adar et al. 2016). In this perspective, our sample is biased from the usual community population, since more than 77% of our participants obtained at least some college education. Another possible explanation is that women with higher education may also have a healthier lifestyle, like more outdoor activities, who may engage in behavioral associated high exposure. But, we did not collect this information. However, as indicated in the 10-year follow-up study of MESA Air, the information related to behavior is very difficult to models. In their time weighted exposure estimation, they did not find an association with CAC or CIMT, which may due to the uncertainty of the activity related the information (Kaufman, Adar et al. 2016).

One of the limitation in our study is the gap between exposure assessment and outcomes collection, which was about 5 – 9 years. This gap is not only for the air pollutant exposure assessment, but also for the other biomarkers whichever relied on biospecimen collection. However, the data we have for air pollution exposure at SWAN visits 3 – 7, when the U.S. Environmental Protection Agency started collecting $PM_{2.5}$ level national wide, and during those years, the exposure levels were much higher than recent years (U.S. Environmental Protection Agency 2016). Thus, we can catch the available exposure data at a relatively higher level in their lives. Another potential limitation is that monitor is used for exposure estimation without any spatiotemporal modeling. The modeled data can overcome the missing values, and may add exposure variation if the spatial variance was well modelled. But, most of the modeling methods

have limitations and uncertainties (Rao, Galmarini et al. 2011, Venkatram 2015). Moreover, if the extrapolation of the exposure modeling to a period with extensive missing data from the monitors, the estimation may not be valid. Our outcome of CIMT was only collected at CCA segment, which may not be the optimal biomarker of subclinical atherosclerosis. Rivera et al. revealed that there was a stronger association between 10-year NO_2 exposure and CIMT as mean of 6 segments than CCA segment only (Rivera, Basagaña et al. 2012). Plaque and CIMT thickening is more likely to occurre in the internal carotid artery and the bulb segment, arterial areas exposed to suffered from greater turbulence flow and sheer stress (Ku, Giddens et al. 1985, Plesniak and Peterson 2004, Dhawan, Avati Nanjundappa et al. 2010).

We have the advantage of studying air pollution exposure during the menopausal transition in women as a predictor of their post-menopausal subclinical atherosclerosis. SWAN collected extensive data of CVD risk factors of these women during the menopausal transitioning, which allowed us to answer the question of the effect of chronic long-term $PM_{2.5}$ exposure on CCA IMT thickening in post-menopause. We uniquely established the association of the cumulative chronic long-term $PM_{2.5}$ exposure for 5 years and subclinical atherosclerosis. Most other studies were only able to observe a rather short-term exposure period, or the exposure estimates were based on the spatial modeling of exposure during a 1 – 2-year period (Künzli, Jerrett et al. 2005, Bauer, Moebus et al. 2010, Lenters, Uiterwaal et al. 2010, Perez, Wolf et al. 2015). As atherosclerosis is a life-long process (Künzli, Perez et al. 2011), the long-term exposure would be more meaningful in establishing the relationship with atherosclerosis.

4.6 CONCLUSION

In conclusion, chronic long-term exposure to $PM_{2.5}$ in early mid-life independently contributes to atherosclerosis as measured by mean of maximum CCA at later mid-life in multi-ethnic population based cohort of women. The findings from this study extend the evidence in the current literature that chronic long-term exposure to $PM_{2.5}$ is harmful to heart health.

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4.8 TABLES AND FIGURES

Table 4-2 Characteristics by cumulative yearly PM2.5 exposure quartiles (N=1188)

	$PM_{2.5}$ ($\mu g/m^3$)				p-trend
	9.8 – 13.58 (n=297)	13.58 – 15.34 (n=297)	15.34 – 16.14 (n=297)	16.14 – 20.84 (n=297)	
Ozone (ppb)	25.0 (6.2)	34.6 (3.3)	33.2 (3.3)	33.7 (4.2)	<.001
Age* (years)	60.1 (2.6)	59.3 (2.7)	59.3 (2.6)	60.0 (2.7)	.638
Race** (%)					<.001
White	43.1	57.6	47.1	45.1	
Black	3.4	38.1	48.5	35.7	
Chinese	53.6	0	0	0	
Hispanic	0	4.4	4.4	19.2	
Ever Smoking* (%)	24.0	41.9	39.0	49.5	<.001
Education** (%)					0.919
≤ High School	20.9	24.8	23.7	22.3	
Some College/College	51.2	55.2	49.5	53.3	
> College	28.0	20.0	26.8	24.4	
Pay for Basis* (%)					<.001
Consistently Hard	17.5	38.1	36.7	38.4	
Mixed	29.6	27.6	24.6	25.9	
Consistently not hard	52.9	34.3	38.7	35.7	
Menopause Status* (%)					.389
Pre/Peri-menopause	1.7	2.7	2.4	2.4	
Post-menopause	91.6	86.9	86.5	87.9	
Other	6.7	10.4	11.1	9.8	
Ever hormone users (%)	42.1	43.4	43.8	43.8	.675
BMI	25.6 (5.8)	31.5 (7.7)	30.2 (6.7)	30.1 (6.4)	<.001
SBP (mmHg)	111.2 (11.4)	118.7 (15.5)	122.0 (15.7)	122.6 (13.5)	<.001
DBP (mmHg)	71.0 (7.5)	72.1 (8.7)	75.6 (9.1)	77.1 (7.9)	<.001
Hypertension*(%)	48.5	70.7	76.1	76.4	<.001
Hypertension Medication*(%)	33.3	55.6	55.6	60.6	<.001
Cholesterol (mg/dL)	203.2 (31.8)	201.7 (33.8)	197.9 (33.7)	204.2 (32.7)	.933
LDL (mg/dL)	115.8 (26.3)	118.8 (29.7)	116.5 (29.8)	122.5 (30.9)	.022
HDL (mg/dL)	62.3 (15.0)	58.1 (14.1)	56.9 (14.2)	56.9 (14.0)	-<.001
Triglyceride (mg/dL)	103.9 (76.7, 151.6)	101.4 (77.9, 151.5)	104.4 (80.4, 143.5)	108.0 (85.3, 146.0)	.616
Lipids Lowering Medication (%)	21.9	36.0	38.1	37.7	<.001

Table 4-2 Continued

Glucose (mg/dL)	88.9 (83.8, 94.3)	87.6 (83.3, 95.8)	88.6 (84.1, 95.8)	88.7 (83.8, 97.0)	.281
Insulin (uIU/mL)	9.3 (7.5, 12.4)	11.2 (8.3, 16.9)	11.4 (8.6, 15.9)	11.5 (8.5, 16.9)	<.001
Diabetes* (%)	7.4	15.8	16.5	19.2	<.001
Diabetic medication (%)	7.1	15.8	14.8	18.2	<.001
hsCRP(mg/L)	1.2 (0.6, 2.9)	2.9 (1.0, 7.7)	2.9 (1.2, 5.9)	2.9 (1.3, 6.3)	<.001
tPA (ng/dL)	6.5 (4.8, 8.4)	7.6 (5.5, 9.4)	7.4 (5.8, 9.6)	7.8 (6.0, 10.1)	<.001
PAI (ng/dL)	14.4 (7.3, 26.1)	19.8 (10.9, 38.2)	19.1 (10.9, 35.1)	22.2 (11.9, 35.5)	<.001
Avg. CCA IMT*(mm)	0.76 (0.11)	0.80 (0.11)	0.80 (0.12)	0.81 (0.13)	<.001
Max. CCA IMT*(mm)	0.89 (0.13)	0.94 (0.13)	0.93 (0.14)	0.95 (0.15)	<.001
IAD*(mm)	7.12 (0.72)	7.24 (0.72)	7.24 (0.66)	7.20 (0.67)	.175
Plaque* (%)	47.8	41.9	40.4	40.4	-.065
Plaque index* (%)					-.025
0	52.2	58.1	59.6	59.6	
1-2	29.3	28.7	27.6	29.6	
3+	18.5	13.2	13.8	10.8	

* Data was shown as at carotid visits (SWAN visits 12/13); ** Data was shown as at baseline; All the other information was cumulative of visits 3 – 7 as corresponding to the available air pollution data

Table 4-3 Spearman correlation between major time-weighted yearly cumulative air pollutants and IMT measures

	$PM_{2.5}$ ($\mu g/m^3$)	O_3 (ppb)	Mean CIMT*(mm)	Maximum CIMT*(mm)	IAD*(mm)
$PM_{2.5}$ ($\mu g/m^3$)	1.000	.576	.150	.156	.066
O_3 (ppb)	--	1.000	.088	.060	.004
Mean CIMT*(mm)	--	--	1.000	.958	.571
Maximum CIMT*(mm)	--	--	--	1.000	.576
IAD*(mm)	--	--	--	--	1.000

* Data was shown as at carotid visits (SWAN visits 12/13); All the other information was cumulative of visits 3 – 7 as corresponding to the available air pollution data

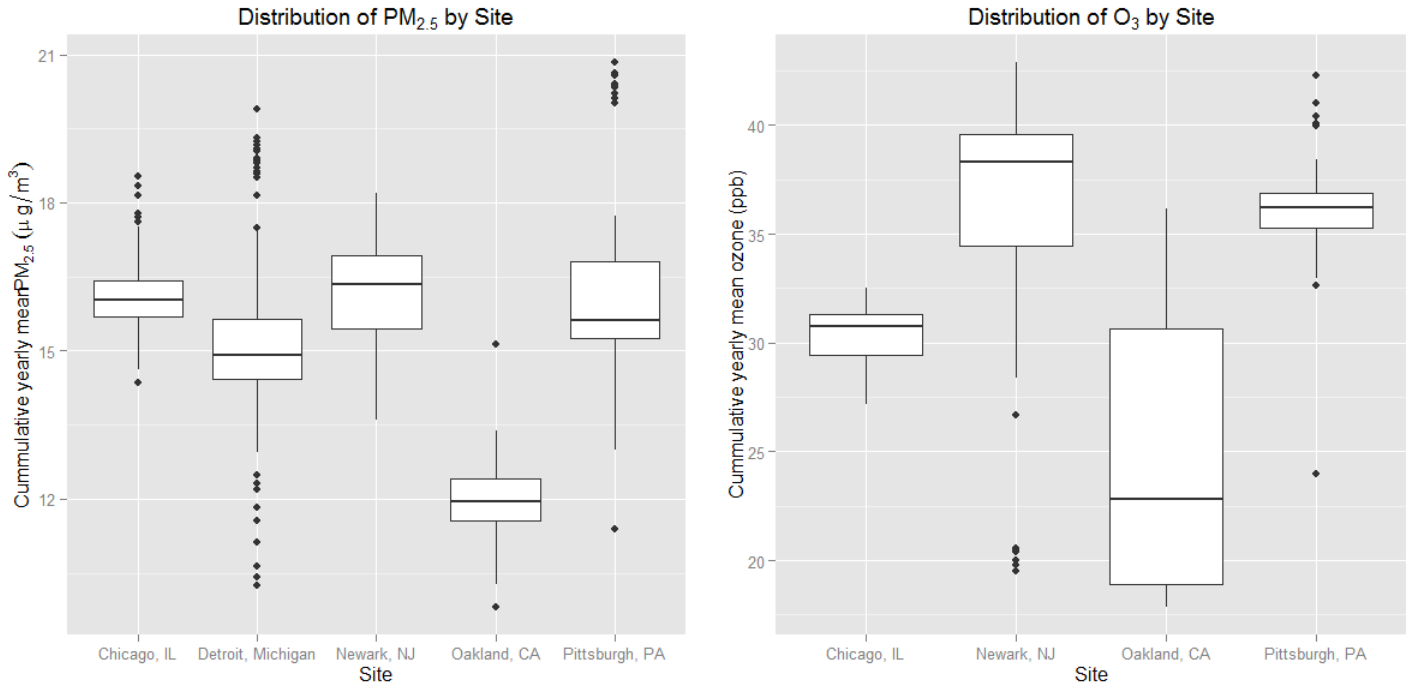

Figure 4-1 Yearly Cumulative Exposure level of $PM_{2.5}$ and O_3 by site

Table 4-4 Association between $PM_{2.5}$ and O_3 and mean common carotid artery intima-media thickness (CCA IMT)

Models	Mean CCA IMT (μm)
$PM_{2.5}$ ($\mu g/m^3$)	
Model 1	9.59 (3.19 – 15.98) ***
Model 2a	7.57 (1.31 – 13.82) **
Model 2b	5.59 (-0.58 – 11.75) *
Model 3	3.22 (-2.83 – 9.26)
Model 2b + SBP	3.48 (-2.56 – 9.53)
Model 2b + HTN†	5.44 (-0.67 – 11.56) *
Model 2b + hsCRP	5.45 (-0.71 – 11.61) *
Model 2b + tPA	5.64 (-0.54 – 11.82) *
Model 2b + PAI-1	5.67 (-0.50 – 11.84) *
O_3 (ppb)#	
Model 1	-0.86 (-2.95 – 1.23)
Model 2a	-0.20 (-2.27 – 1.87)
Model 2b	-0.49 (-2.51 – 1.53)
Model 3	-0.65 (-2.63 – 1.33)

Note: Model 1 adjust for site; model 2a further adjust for age, race, education, and longitudinal financial strain; model 2b additionally adjust for BMI, smoking, cholesterol, HDL, triglyceride, menopause status, hormone use, fasting glucose, diabetes medication, and blood pressure medication; model 3 adjusting for potential mediators: systolic blood pressure (SBP), C-reactive protein, tPA and PAI-1.

* Marginal significant, $0.05 \leq p < 0.1$, ** $p < 0.05$, *** $p < 0.01$

† model 2b + HTN, but not adjust for blood pressure medication

Models included O_3 excluding site Michigan as the extensive missing values

Table 4-5 Association between $PM_{2.5}$ and O_3 and mean of the maximum common carotid artery intima-media thickness (CCA IMT)

Models	Mean of maximum CCA IMT (μm)
$PM_{2.5}$ ($\mu g/m^3$)	
Model 1	12.57 (5.21 – 19.93) ****
Model 2a	10.46 (3.29 – 17.63) ***
Model 2b	8.03 (1.01 – 15.05) **
Model 3	5.02 (-1.86 – 11.90)
Model 2b + SBP	5.40 (-1.48 – 12.28)
Model 2b + HTN†	7.90 (0.94 – 14.86) **
Model 2b + hsCRP	7.86 (0.84 – 14.87) **
Model 2b + tPA	8.01 (0.97 – 15.04) **
Model 2b + PAI-1	8.11 (1.08 – 15.14) **
O_3 (ppb)#	
Model 1	-1.38 (-3.81 – 1.05)
Model 2a	-0.51 (-2.90 – 1.88)
Model 2b	-0.92 (-3.23 – 1.38)
Model 3	-1.09 (-3.34 – 1.15)

Note: Model 1 adjust for site; model 2a further adjust for age, race, education, and longitudinal financial strain; model 2b additionally adjust for BMI, smoking, cholesterol, HDL, triglyceride, menopause status, hormone use, fasting glucose, diabetes medication, and blood pressure medication; model 3 adjusting for potential mediators: systolic blood pressure (SBP), C-reactive protein, tPA and PAI-1.

* Marginal significant, $0.05 \leq p < 0.1$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$

† model 2b + HTN, but not adjust for blood pressure medication

Models included O_3 excluding site Michigan as the extensive missing values

Table 4-6 Association between $PM_{2.5}$ and O_3 and mean inter-adventitial diameter (IAD)

Models	Mean IAD (μm)
$PM_{2.5}$ ($\mu g/m^3$)	
Model 1	42.48 (6.77 – 78.19) **
Model 2a	35.28 (-0.01 – 70.57) *
Model 2b	21.18 (-12.60 – 54.96)
Model 3	8.99 (-24.26 – 42.26)
O_3 (ppb)#	
Model 1	-2.36 (-14.11 – 9.39)
Model 2a	0.16 (-11.60 – 11.91)
Model 2b	-0.47 (-11.67 – 10.72)
Model 3	-2.11 (-13.16 – 8.93)

Note: Model 1 adjust for site; model 2a further adjust for age, race, education, and longitudinal financial strain; model 2b additionally adjust for BMI, smoking, cholesterol, HDL, triglyceride, menopause status, hormone use, fasting glucose, diabetes medication, and blood pressure medication; model 3 adjusting for potential mediators: systolic blood pressure (SBP), C-reactive protein, tPA and PAI-1.

* Marginal significant, $0.05 \leq p < 0.1$, ** $p < 0.05$

Models included O_3 excluding site Michigan as the extensive missing values

Table 4-7 Association between $PM_{2.5}$ and O_3 and plaque presence

Models	Odd of Plaque Presence
$PM_{2.5}$ ($\mu g/m^3$)	
Model 1	0.98 (0.88 – 1.09)
Model 2a	1.00 (0.89 – 1.11)
Model 2b	0.98 (0.87 – 1.10)
Model 3	0.97 (0.86 – 1.09)
O_3 (ppb)#	
Model 1	1.01 (0.97 – 1.05)
Model 2a	1.01 (0.97 – 1.05)
Model 2b	1.01 (0.97 – 1.05)
Model 3	1.01 (0.97 – 1.05)

Note: Model 1 adjust for site; model 2a further adjust for age, race, education, and longitudinal financial strain; model 2b additionally adjust for BMI, smoking, menopause status, hormone use, cholesterol, HDL, fasting glucose, diabetes medication use, and blood pressure medication use; model 3 adjusting for potential mediators: systolic blood pressure (SBP), C-reactive protein, tPA and PAI-1.

Models included O_3 excluding site Michigan as the extensive missing values

Table 4-8 Association between $PM_{2.5}$ and O_3 and plaque index

Models	Odds of Plaque index		
	0	1-2	> 2
$PM_{2.5}$ ($\mu g/m^3$)			
Model 1	Reference	0.99 (0.88 – 1.12)	0.96 (0.82 – 1.12)
Model 2a	Reference	1.01 (0.89 – 1.14)	0.99 (0.85 – 1.16)
Model 2b	Reference	1.01 (0.89 – 1.15)	0.93 (0.79 – 1.10)
Model 3	Reference	1.01 (0.88 – 1.15)	0.91 (0.76 – 1.08)
O_3 (ppb)#			
Model 1	Reference	1.03 (0.99 – 1.07)	0.98 (0.93 – 1.03)
Model 2a	Reference	1.03 (0.98 – 1.07)	0.98 (0.93 – 1.03)
Model 2b	Reference	1.02 (0.98 – 1.07)	0.98 (0.93 – 1.04)
Model 3	Reference	1.03 (0.98 – 1.07)	0.98 (0.92 – 1.03)

Note: Model 1 adjust for site; model 2a further adjust for age, race, education, and longitudinal financial strain; model 2b additionally adjust for BMI, smoking, menopause status, hormone use, cholesterol, HDL, fasting glucose, diabetes medication use, and blood pressure medication use; model 3 adjusting for potential mediators: systolic blood pressure (SBP), C-reactive protein, tPA and PAI-1.

† Michigan site was excluded, since there were only 4 participants with O_3 exposure level available.

Models included O_3 excluding site Michigan as the extensive missing values

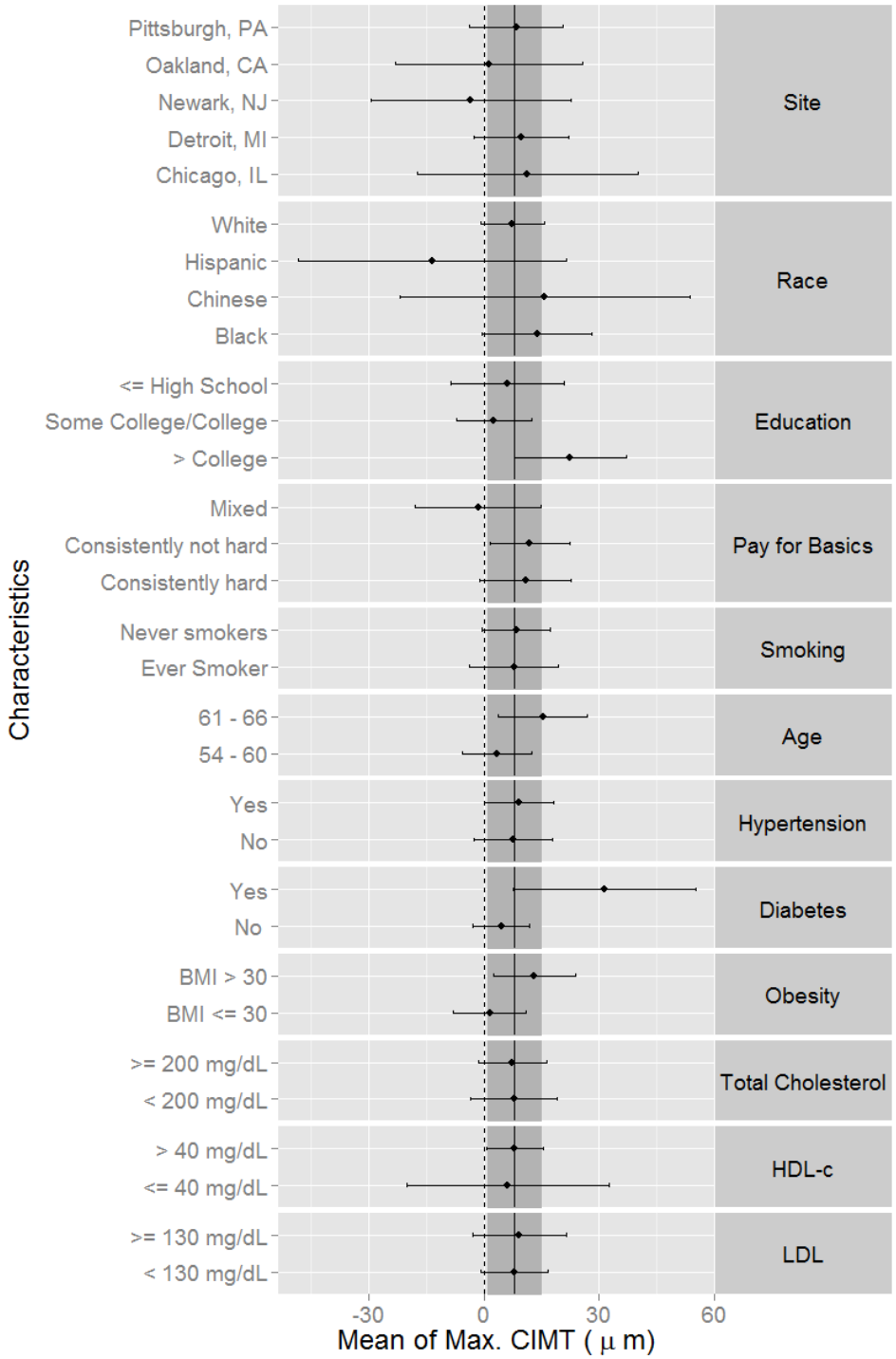

Figure 4-2 Association between $PM_{2.5}$ and mean of the maximum common carotid artery intima-media thickness (CCA IMT), by participant characteristics.

Note: The solid line showed the point estimate of $PM_{2.5}$ and mean of maximum CIMT in the fully adjusted model, and the shaded area showed the 95% CI of this estimate. The dash line indicated 0, which is the null effect of CIMT thickening.

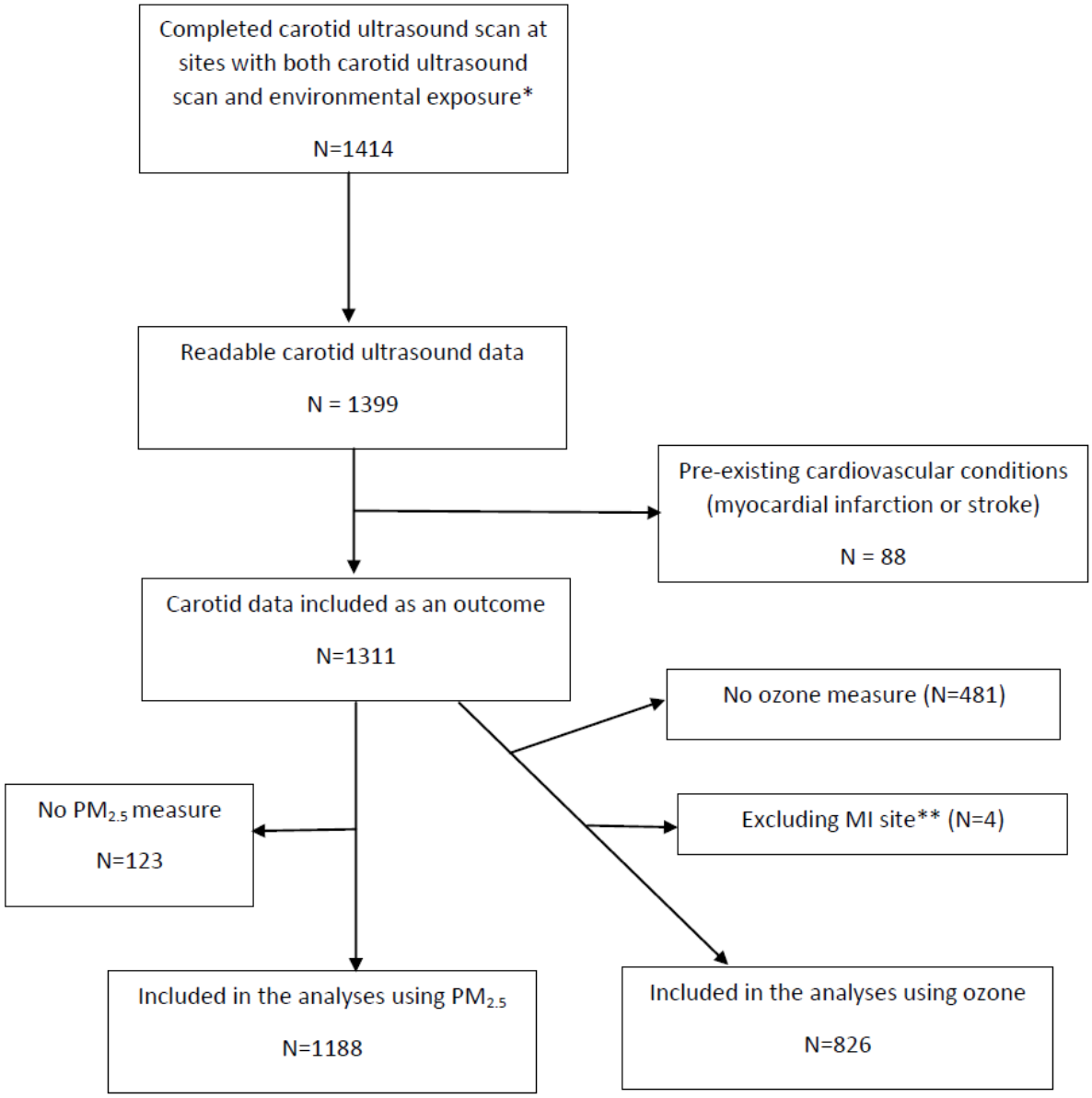

Figure S 4-1 Population included in the analyses

* Five sites included in this analysis: Detroit, MI; Oakland, CA; Pittsburgh, PA; Chicago, IL; and Newark, NJ

** Detroit, MI site only got 4 participants with year-long ozone estimation. So, we will exclude the whole site with the analyses including ozone.

Table S 4-1 Two-pollutant model of $PM_{2.5}$ and O_3 and mean common carotid artery intima-media thickness (CCA IMT) ǂ

Models		Mean CCA IMT (μm)
Model 1	$PM_{2.5}$ (μg/m^3)	7.66 (-1.14 – 16.45) *
	O_3 (ppb)	-0.73 (-2.82 – 1.37)
Model 2a	$PM_{2.5}$ (μg/m^3)	6.15 (-2.51 – 14.80)
	O_3 (ppb)	-0.07 (-2.15 – 2.01)
Model 2b	$PM_{2.5}$ (μg/m^3)	3.16 (-5.32 – 11.65)
	O_3 (ppb)	-0.43 (-2.45 – 1.59)
Model 3	$PM_{2.5}$ (μg/m^3)	2.55 (-5.72 – 10.82)
	O_3 (ppb)	-0.59 (-2.58 – 1.39)

Note: Model 1 adjust for site; model 2a further adjust for age, race, education, and longitudinal financial strain; model 2b additionally adjust for BMI, smoking, cholesterol, HDL, triglyceride, menopause status, hormone use, fasting glucose, diabetes medication, and blood pressure medication; model 3 adjusting for potential mediators: systolic blood pressure (SBP), C-reactive protein, tPA and PAI-1.

* Marginal significant, $0.05 < p \leq 0.1$

ǂ Michigan site was excluded, since there were extensive missing values with O_3 exposure.

Table S 4-2 Two-pollutant model of $PM_{2.5}$ and O_3 and mean of maximum common carotid artery intima-media thickness (CCA IMT) ǂ

Models		Mean of maximum CCA IMT (μm)
Model 1	$PM_{2.5}$ (μg/m^3)	11.61 (1.53 – 21.70) **
	O_3 (ppb)	-1.23 (-3.66 – 1.20)
Model 2a	$PM_{2.5}$ (μg/m^3)	10.19 (0.32 – 20.05) **
	O_3 (ppb)	-0.36 (-2.75 – 2.04)
Model 2b	$PM_{2.5}$ (μg/m^3)	6.59 (-2.99 – 16.18)
	O_3 (ppb)	-0.84 (-3.15 – 1.46)
Model 3	$PM_{2.5}$ (μg/m^3)	5.53 (-3.79 – 14.84)
	O_3 (ppb)	-1.09 (-3.34 – 1.17)

Note: Model 1 adjust for site; model 2a further adjust for age, race, education, and longitudinal financial strain; model 2b additionally adjust for BMI, smoking, cholesterol, HDL, triglyceride, menopause status, hormone use, fasting glucose, diabetes medication, and blood pressure medication; model 3 adjusting for potential mediators: systolic blood pressure (SBP), C-reactive protein, tPA and PAI-1.

* Marginal significant, $0.05 \leq p < 0.1$, ** $p < 0.05$

ǂ Michigan site was excluded, since there were extensive missing values with O_3 exposure.

Table S 4-3 Two-pollutant model of $PM_{2.5}$ and O_3 and mean inter-adventitial diameter (IAD) ✝

Models		Mean IAD (μm)
Model 1	$PM_{2.5}$ (μg/m^3)	21.45 (-27.50 – 70.40)
	O_3 (ppb)	-2.02 (-13.80 – 9.76)
Model 2a	$PM_{2.5}$ (μg/m^3)	17.37 (-31.24 – 65.99)
	O_3 (ppb)	-0.61 (-12.32 – 11.09)
Model 2b	$PM_{2.5}$ (μg/m^3)	-5.45 (-52.17 – 41.28)
	O_3 (ppb)	-0.45 (-11.66 – 10.77)
Model 3	$PM_{2.5}$ (μg/m^3)	-11.40 (-57.03 – 34.22)
	O_3 (ppb)	-1.73 (-12.76 – 9.30)

Note: Model 1 adjust for site; model 2a further adjust for age, race, education, and longitudinal financial strain; model 2b additionally adjust for BMI, smoking, cholesterol, HDL, triglyceride, menopause status, hormone use, fasting glucose, diabetes medication, and blood pressure medication; model 3 adjusting for potential mediators: systolic blood pressure (SBP), C-reactive protein, tPA and PAI-1.
✝ Michigan site was excluded, since there were extensive missing values with O_3 exposure.

Table S 4-4 Two-pollutant model of $PM_{2.5}$ and O_3 and plaque presence ✝

Models		Odd of Plaque Presence
Model 1	$PM_{2.5}$ (μg/m^3)	0.97 (0.84 – 1.13)
	O_3 (ppb)	1.01 (0.97 – 1.05)
Model 2a	$PM_{2.5}$ (μg/m^3)	0.97 (0.83 – 1.13)
	O_3 (ppb)	1.01 (0.97 – 1.05)
Model 2b	$PM_{2.5}$ (μg/m^3)	0.92 (0.79 – 1.09)
	O_3 (ppb)	1.00 (0.97 – 1.04)
Model 3	$PM_{2.5}$ (μg/m^3)	0.91 (0.78 – 1.08)
	O_3 (ppb)	1.00 (0.97 – 1.05)

Note: Model 1 adjust for site; model 2a further adjust for age, race, education, and longitudinal financial strain; model 2b additionally adjust for BMI, smoking, menopause status, hormone use, cholesterol, HDL, fasting glucose, diabetes medication use, and blood pressure medication use; model 3 adjusting for potential mediators: systolic blood pressure (SBP), C-reactive protein, tPA and PAI-1.
✝ Michigan site was excluded, since there were extensive missing values with O3 exposure.

Table S 4-5 Two-pollutant model of $PM_{2.5}$ and O_3 and plaque index ☨

Models		Odds of Plaque index		
		0	1-2	> 2
Model 1	$PM_{2.5}$ ($\mu g/m^3$)	Reference	0.95 (0.80 – 1.12)	1.05 (0.84 – 1.31)
	O_3 (ppb)	Reference	1.03 (0.99 – 1.07)	0.98 (0.93 – 1.03)
Model 2a	$PM_{2.5}$ ($\mu g/m^3$)	Reference	0.94 (0.79 – 1.12)	1.05 (0.83 – 1.31)
	O_3 (ppb)	Reference	1.03 (0.98 – 1.07)	0.98 (0.93 – 1.04)
Model 2b	$PM_{2.5}$ ($\mu g/m^3$)	Reference	0.92 (0.77 – 1.10)	0.94 (0.74 – 1.19)
	O_3 (ppb)	Reference	1.02 (0.98 – 1.07)	0.98 (0.93 – 1.04)
Model 3	$PM_{2.5}$ ($\mu g/m^3$)	Reference	0.91 (0.76 – 1.09)	0.92 (0.72 – 1.17)
	O_3 (ppb)	Reference	1.02 (0.98 – 1.07)	0.97 (0.92 – 1.03)

Note: Model 1 adjust for site; model 2a further adjust for age, race, education, and longitudinal financial strain; model 2b additionally adjust for BMI, smoking, menopause status, hormone use, cholesterol, HDL, fasting glucose, diabetes medication use, and blood pressure medication use; model 3 adjusting for potential mediators: systolic blood pressure (SBP), C-reactive protein, tPA and PAI-1.

☨ Michigan site was excluded, since there were extensive missing values with O_3 exposure.

5.0 ASSOCIATION BETWEEN RESIDENTIAL EXPOSURE TO $PM_{2.5}$ AND OZONE AND PROGRESSION OF SUBCLINICAL ATHEROSCLEROSIS AMONG WOMEN TRANSITIONING THROUGH MENOPAUSE: THE STUDY OF WOMEN'S HEALTH ACROSS THE NATION

5.1 ABSTRACT

Objective: This paper aims to explore the association between long-term air pollution and progression of subclinical atherosclerosis among women transitioning through menopause from the Study of Women's Health Across the Nation (SWAN).

Methods: Carotid duplex ultrasonography was performed twice, 2.2 year apart, in participants from SWAN Heart, an ancillary study of SWAN carried out at the Pittsburgh and Chicago sites. Mean of the average and the maximum carotid intima-media thickness (CIMT) and plaque burden were assessed throughout the common, bulb and internal carotid artery. Participants' residential addresses were also collected at these visits. The yearly-mean exposure to $PM_{2.5}$ and ozone (O_3) were generated based on monitor data within 20 km of the participants' home. The effect of exposure to air pollutants during the follow-up on progression of CIMT was examined using linear mixed effect models. The associations between yearly mean cumulative exposure to air pollutants during the follow-up and progression of plaque presence and plaque index, a measure of extent of plaque, were estimated using logistic regression.

Results: This study included 417 (257 White and 160 Black) women with mean age of 51 years (SD = 2.8) at baseline. Baseline mean CIMT was 677.9 μm (±93.7). In the primary analysis, a 1 $\mu g/m^3$ higher yearly mean exposure to $PM_{2.5}$ during the follow-up was associated with a 4.28 (95% CI: 0.02 – 8.54) μm per year increase of mean of maximum CIMT progression, after adjusting for confounders (site, age, race/ethnicity, education, financial strain, BMI, smoking, triglycerides, LDL-c, and HDL-c levels, lipid lowering medication, hypertension medication, menopause status, hormone replacement therapy, diabetes and family history of CVD or stroke). The effect size did not change substantially after adjusting for the potential mediators: systolic blood pressure, C-reactive protein, tissue-type plasminogen activator antigen, and plasma plasminogen activator inhibitor 1. No association was found between O_3 and progression of CIMT or plaque. A 1 $\mu g/m^3$ higher yearly mean exposure to $PM_{2.5}$ during the follow-up contributed to about 30% (95% CI: 3% - 65%) chance of increase in plaque index progression in both single and two-pollutant models adjusting for socioeconomic factors, but not in the fully adjusted model.

Conclusion: $PM_{2.5}$ independently contributed to subclinical atherosclerosis progression among women transitioning through menopause. This study provides additional evidence that levels of air pollution may have significant deleterious effects on women's CVD risk in midlife.

Keywords: $PM_{2.5}$, ozone (O_3), Subclinical Atherosclerosis, carotid intima-media thickness (CIMT) progression and plaque progression

5.2 INTRODUCTION

Air pollution is ubiquitous, and exists in our everyday environment. During the past two decades, there have been a growing number of studies demonstrating that air pollution has a deleterious effect on health (Lim, Vos et al. 2012), and the most notable health outcomes are cardiovascular (CVD) and pulmonary disease (Brook, Rajagopalan et al. 2010, Franchini and Mannucci 2012, Atkinson, Carey et al. 2013, Franklin, Brook et al. 2015). Franklin et al. estimated that air pollution may account for 10 – 25% risk of coronary heart disease that is not explained by traditional risk factors. However, the effect of long-term exposure of air pollution and the mechanisms leading to CVD are unclear, which may be through a sub-clinical atherosclerosis pathway. Current research suggests there are inflammatory reactions to Air toxics such as PM2.5 and ozone leading to increased subclinical atherosclerosis (Brook and Rajagopalan 2010, Araujo 2011, Delfino, Staimer et al. 2011).

Carotid intima-media thickness (CIMT) measured via B-mode ultrasound reflects the carotid artery wall thickness by capturing the thickness between the intimal-luminal and medial-adventitial interfaces. It is a surrogate biomarker of atherosclerosis (Bauer, Caviezel et al. 2012), that predicts CVD and stroke events in population based cohorts (Chambless, Folsom et al. 2000, Bots, Evans et al. 2003, Bauer, Caviezel et al. 2012, van den Oord, Sijbrands et al. 2013). Researchers reported that change in CIMT may be a valid predictor of vascular events (Geerts, Bots et al. 2008, Polak, Pencina et al. 2011, Baldassarre, Veglia et al. 2013, Okayama, Mita et al. 2013, Naqvi and Lee 2014), and a surrogate measure for intervention studies (Bots, Evans et al. 2003, Hodis, Mack et al. 2016). CIMT is one of the most established subclinical biomarkers of atherosclerosis in population based studies, as well as in the domain of occupational and environmental health settings (Hoffmann 2015).

Plaque is a direct measure of atherosclerosis and its presence in the carotid arteries may better predict CVD risk compared to CIMT alone (Inaba, Chen et al. 2012). Quantitative measures of plaque may be an even better predictor than plaque presence (Naqvi and Lee 2014). Few studies have considered plaque progression, and have been limited to patients who already had significant atherosclerosis (Spence, Eliasziw et al. 2002, Wannarong, Parraga et al. 2013, van Engelen, Wannarong et al. 2014). One community study conducted in Taiwan observed that progression of plaque scores can predict CVD adjusted for age and sex, but not in the models considering traditional CVD risk factors (Chen, Jeng et al. 2016).

The cross-sectional relationship between exposure to one-year air pollution and atherosclerosis has been examined in several studies in different populations, and most of these studies established a positive relationship (Künzli, Jerrett et al. 2005, Bauer, Moebus et al. 2010, Lenters, Uiterwaal et al. 2010, Breton, Wang et al. 2012, Rivera, Basagaña et al. 2012, Tonne, Yanosky et al. 2012, Kim, Sheppard et al. 2014, Perez, Wolf et al. 2015, Su, Hwang et al. 2015). To date, there are only a few published studies addressing the longitudinal association between $PM_{2.5}$ (particulate matter with aerodiameter less than 2.5 μm) and ozone (O_3) and progression of atherosclerosis and most of them are from the same cohort. In the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air) study (Adar, Sheppard et al. 2013), a 2.5 $\mu g/m^3$ increase in mean follow-up $PM_{2.5}$ was associated with a 5.0 μm (95% CI: 2.6 – 7.4) greater CIMT progression. However, a more recent ten-year follow-up of the study did not find an association with CIMT progression, but did find an association with coronary artery calcification (CAC) progression (Kaufman, Adar et al. 2016). A newly published abstract from MESA Air study reported that a ten-year follow-up of O_3 exposure was associated with atherosclerosis progression using both CIMT and plaque as outcomes (Wang, Sheppard et al. 2016). One of the

few studies evaluating plaque progression did not find any significant associations with air pollutants and either CIMT progression or plaque progression, although the study was in a region of very low levels of pollutants (Gan, Allen et al. 2014). When stratified by race, they observed that only Chinese who lived near roads had an increase of 1.12 mm^2 (95% CI: 0.30 – 2.12) annual progression in plaque area. The heterogeneous findings of these studies suggest that more evidence is needed to ascertain the association between air pollution and progression of atherosclerosis.

We had the opportunity to examine the association between exposure to $PM_{2.5}$ and O_3 and progression of atherosclerosis over a 2.2-year period, utilizing both CIMT and plaque as biomarkers of atherosclerosis in a cohort of middle-aged black and white women. We hypothesized that women who are exposed to higher levels of $PM_{2.5}$ and O_3 over time would have a higher progression of CIMT and plaque index, a measure of plaque severity.

5.3 METHODS

5.3.1 Study Population

The Study of Women's Health Across the Nation (SWAN) is a community-based multi-center multi-ethnic cohort study designed to characterize women's health transitioning through menopause. The SWAN study was conducted at seven sites across the U.S. beginning in 1996. There were 3302 women enrolled at baseline at age 42 – 52 years. At the time of enrollment, these women had an intact uterus and at least one ovary, were not pregnant or breast-feeding, had menstruated within the past 3 months, and were not using oral contraceptives or in hormone

therapy. The SWAN Heart, an ancillary study of SWAN, focused on the heart health among these women by additionally obtaining subclinical measures of atherosclerosis at two SWAN visits on average of 2.2 years apart. The baseline carotid ultrasound measurements were obtained in participants attending SWAN visits 4 – 7 (2001– 2004). These participants were invited back to obtain a follow-up ultrasound measure at a subsequent visits corresponding to SWAN visits 6 – 9 (2002 – 2006) (Table S 5-1). The two SWAN Heart study sites, Chicago and Pittsburgh, both recruited White and Black women. The exclusion criteria for SWAN Heart were that among the women who had a baseline visit, if they were pregnant at the time, they were ineligible; among the women who did not have a baseline visit, if they were pregnant, had coronary artery bypass grafting, percutaneous transluminal coronary angioplasty, peripheral vascular surgery, or endarterectomy; had a hysterectomy or bilateral oophorectomy; had a history of cardiovascular disease, CHF, Stroke or TIA; had treated diabetes; or had taken female hormones in the past 3 months. The study was approved by the Institutional Review Board at each site. Written informed consents were obtained by all of the participants.

5.3.2 Exposure to $PM_{2.5}$ and O_3

Air pollution exposure was assessed by the Air Pollution Study, another ancillary study of SWAN. This study determined the residential air pollutant exposure levels of SWAN participants of visits 3 – 7. This study group collected the residential address of participants of visits 3 – 7. All the addresses were geo-coded. Daily $PM_{2.5}$ and O_3 values were retrieved from US EPA Air Quality System DataMart (US EPA 2015). Exposure to $PM_{2.5}$ and O_3 was determined by one monitor if it was located within 20 km from the participant's address. Annual exposure to these pollutants was defined as 360 days before the study visit. Detailed methods of

exposure assessment and the address geo-coding have been published elsewhere (Ostro, Malig et al. 2014, Green, Broadwin et al. 2016). These data were collected for a one-year exposure period prior to each of the SWAN visits 3 – 7. The corresponding annual mean exposure of $PM_{2.5}$ and O_3 before SWAN Heart baseline was extracted in the analysis. An annualized mean cumulative level of each air pollutant ($PM_{2.5}$ and O_3) was calculated based on the mean of the yearly exposure prior to the visits from SWAN Heart baseline to SWAN Heart follow-up carotid visit.

5.3.3 Assessment of CIMT and plaque

The carotid arteries were scanned via B-mode ultrasound by centrally trained and experienced sonographer. In Chicago, Hewlett Packard SONOS 5500 scanner (Hewlett Packard, Andover, MA) was used to collect the images, and in Pittsburgh, Toshiba 270 A (Toshiba American Medical Systems, Tustin, CA) was used. The qualities of the images were comparable between the two machines (Whipple, Lewis et al. 2009, Thurston, Sutton-Tyrrell et al. 2011). Participants were scanned in the supine position with the head turned 45° toward the side opposite the side being examined. The carotid artery was imaged in its long axis with multiple scanning angles (anterior, lateral, and posterior) and the angle of interrogation resulting in the thickest IMT was used for later reading. Images were collected at both left and right side of carotid artery at four locations: two at the common carotid artery (CCA), one at the bulb and one at internal carotid artery (ICA). These images were read using semi-automated edge detection reading software (AMS). The same scanning and reading protocols used in this study has yielded high reproducibility as previously reported (Whipple, Lewis et al. 2009, Thurston, Sutton-Tyrrell et al. 2011). The mean of the average and maximum of these eight segments were calculated as the

major outcomes. Inter-adventitial diameters (AD) were measured directly as the distance from the adventitial-media interface on the near wall to the media-adventitial interface on the far wall of the CCA segment only (El Khoudary, Wildman et al. 2013). Plaque presence, number and grade was assessed during the ultrasound scan in five carotid segments, proximal and distal segments of the CCA, bulb, ICA and external carotid artery (ECA). For each segment, plaque grade was categorized into 4 levels, 0 represented no plaque, 1 represented one small plaque, 2 one medium size plaque or several small plaques, and 3 as a plaque taking up more than 50% diameter of the artery. The sum of plaque grades across all segments generated our major outcome plaque index.

5.3.4 Assessment of other CVD risk factors

Covariates were extracted from the SWAN baseline or SWAN follow-up visit. Self-reported race, education, and financial strain were collected at baseline screening. Financial strain was collected based on the question of how hard to pay for the very basics, which includes living expenses and medical treatment. It was categorized into three classes: very hard, somewhat hard, and not hard at all. The study collected data on traditional CVD risk factors at each follow-up visit. Physical measures of body mass index (BMI), and systolic and diastolic blood pressures (SBP and DBP) were collected using the standard methods. Blood samples were draw after a 12-hour fasting. The samples were sent to the Medical Research Laboratories for analysis. The analyses of high density lipoprotein (HDL-c), low density lipoprotein (LDL-c), total cholesterol, and triglycerides, as well as high-sensitivity C-reactive protein (CRP), tissue-type plasminogen activator antigen (tPA), and plasma plasminogen activator inhibitor 1 (PAI-1) were used

standard methodology, and has been published elsewhere (Thurston, El Khoudary et al. 2011, Thurston, Sutton-Tyrrell et al. 2011).

5.3.5 Statistical Analysis

Since glucose, insulin, and triglycerides were not normally distributed, they were log transformed. Unadjusted associations between $PM_{2.5}$ quartiles and covariates and outcomes were examined by linear regression or logistic regression. Linear mixed effect models were used to estimate the effect of time-varying $PM_{2.5}$ and O_3 exposures on CIMT and AD progression. The associations between the mean exposure during the follow-up and progression of CIMT or AD were assessed by examining the regression coefficient associated with the interaction term of annualized mean of exposure from SWAN Heart baseline to SWAN Heart follow-up and time since baseline (supplement). We adjusted for age at baseline, race, education, financial strain, and covariates at baseline. The progression of plaque was categorized as (a) participants were free of plaque at both visits, or who had plaque at SWAN Heart baseline but plaque free at SWAN Heart follow-up (reference group), and (b) participants who developed plaque at follow-up, or had plaque at both visits. When assessing plaque index progression, we categorized them as (a) participants were free of plaque at both visits, or whose plaque index decrease from SWAN Heart baseline to SWAN Heart follow-up (reference group), and (b) participants who developed plaque at follow, remained the same plaque index or had an increase of plaque index. Logistic regressions were applied to estimate the effect of the annual mean cumulative exposure to $PM_{2.5}$ and O_3 between the SWAN Heart baseline and SWAN Heart follow-up scans and plaque presence or plaque index progression. The annualized mean of air pollutants exposure and continuous covariates from SWAN Heart baseline to SWAN Heart follow-up visits were used in

these models. All the categorical variables were extracted at SWAN Heart baseline. Three models were constructed to establish the association between air pollutant exposures and subclinical atherosclerosis, (1) unadjusted model, controlling for site, (2a) model adjusted for SES/demographic factors: age at baseline, race, education, and financial strain, (2b) fully adjusted model, further adjusted for traditional CVD risk factors: BMI, smoking, triglycerides, LDL-c, HDL-c, lipid-lowering medication, hypertension medication, menopause status, hormone replacement therapy, diabetes and family history of CVD or stroke, and (3) extended model, additionally adjusted for potential mediators; SBP, CRP, tPA and PAI-1. Analyses were performed with SAS (V9.3, SAS Institute, Cary, NC). All tests were 2-sided, $\alpha = 0.05$.

5.4 RESULTS

This study included 417 women who participated in the SWAN Heart study. There were 257 White and 160 Black women with a mean age of 51 years (SD= 2.8) at baseline. The median follow-up time was 2.2 years (range:1.1 – 4.4). The $PM_{2.5}$ exposure level at baseline was 16.5 $\mu g/m^3$ (IQR: 15.7 –17.1), and O_3 exposure level at baseline was 31.9 ppb (IQR: 30.3 – 33.5); the $PM_{2.5}$ exposure level at follow-up was 15.5 $\mu g/m^3$ (IQR: 15.0 – 16.2), and O_3 exposure level at follow-up was 34.4 ppb (IQR: 32.5 – 36.6). The within site exposure level of these two pollutants at baseline and follow-up are shown in Figure 5-1. The progression of mean CIMT was 16.3 (IQR: -1.4 – 33.6) μm per year, the progression of mean of maximum CIMT was 20.8 (IQR: -3.6 – 45.4) μm per year, and the progression of mean AD was 41.4 (IQR: -36.7 – 113.0) μm per year. The per segment progression of CIMT were 15.7 (IQR: -4.4 – 27.8) μm per year, 19.5 (IQR: -10.3 – 45.1) μm per year, and 14.7 (IQR: -21.3 – 41.6) μm per year for CCA, ICA

and BULB, respectively. At baseline, most CVD risk factors were comparable across $PM_{2.5}$ quartiles, except for race/ethnicity, insulin and CRP (Table 5-1). Addresses with lower $PM_{2.5}$ levels were more likely to be residences of White women ($p = 0.038$). Women who lived in the area with higher $PM_{2.5}$ level had significantly higher insulin ($p = 0.017$) and higher CRP ($p = 0.015$). Women exposed to higher $PM_{2.5}$ levels tended to have higher blood pressure ($p = 0.063$), and lower HDL-c ($p = 0.065$). We also observed that at baseline, women who lived in the areas with higher $PM_{2.5}$ exposure had thinner bulb CIMT ($p = 0.023$).

In the primary analysis, a 1 $\mu g/m^3$ higher yearly mean exposure to $PM_{2.5}$ during the follow-up was associated with 2.25 (95% CI: -0.61 – 5.12) μm per year increase of mean CIMT progression; and was associated with a 4.28 (95% CI: 0.02 – 8.54) μm per year increase of mean of maximum CIMT progression, after adjusting for traditional CVD risk factors and confounders. In the per segment analyses, we did not discover any significant association between yearly mean exposure to $PM_{2.5}$ during the follow-up and progression of CCA and ICA CIMT. The association between yearly mean exposure to $PM_{2.5}$ during the follow-up and bulb CIMT was much stronger. Women with exposure to 1 $\mu g/m^3$ higher yearly mean exposure to $PM_{2.5}$ during the follow-up at their residences would have a 6.54 (95% CI: 0.49 – 12.24) μm per year higher BULB CIMT progression (Table 5-3). In the extended model, further adjustment for SBP, CRP, PAI-1 and tPA did not change the association between $PM_{2.5}$ and mean CIMT appreciably. In the model adjusting for the baseline O_3, the association was slightly lower compared to the single pollutant model (Table S 5-4). There was no association between O_3 exposure and mean of the average or the maximum CIMT observed (Table 5-2). Neither pollutant was related to inter-adventitial diameter progression.

There was no association between $PM_{2.5}$ and O_3 and plaque presence progression in either the single pollutant model, or two-pollutant model. $PM_{2.5}$ had an impact on plaque index progression after adjusting for SES/demographic factors in both single and two-pollutant models. Each 1 $\mu g/m^3$ higher yearly mean exposure to $PM_{2.5}$ during the follow-up at the residence was related with about 30% (95%CI: 3% - 65%) higher chance to have the same plaque index or develop more severe plaque index. The relationship between $PM_{2.5}$ and progression of plaque index is only marginally associated in the fully adjusted model in the two-pollutant model ($p = 0.080$). This association was not observed in the fully adjusted model between O_3 index progression in either single- or two-pollutant model (Table 5-4 and Table 5-5).

5.5 DISCUSSION

In this prospective study, exposure to higher residential ambient $PM_{2.5}$ level was associated with accelerated atherosclerosis among midlife women, as shown by both biomarkers, CIMT and plaque index. However, no effect of O_3 on subclinical atherosclerosis was observed.

This is the first study focused on air pollution and atherosclerosis among women transitioning through the menopause. The menopause is a very special stage of women's life, and is associated with heart health. Premenopausal women have a lower risk of coronary heart disease (CHD) than men, but after menopause, women's risk increased (Maas and Appelman 2010). The increasing CVD risk across the menopause stages has been observed using these subclinical measures of CIMT. In the Healthy Women Study, pre-menopausal women had a lower CIMT compared to post-menopausal women, and menopause status was a predictor of higher CIMT among these women (Sutton-Tyrrell, Lassila et al. 1998). In a prior analysis of the

SWAN Heart population, postmenopausal women had a greater progression of CIMT and AD compared to premenopausal women (El Khoudary, Wildman et al. 2013). In this study, most women were in the early pre/early peri-menopause status (56.8%), when their risk of CVD may change dramatically; and therefore, have an accelerated change in CIMT. Investigating women at this stage of their life may account for a greater proportion of their heart health change from pre/peri- to post-menopause. In our model, we did not find any significant results with menopause status, due to the homogeneity of the population (e.g., similar stage of their life and short follow-up).

Among these women, we found that 1 $\mu g/m^3$ higher $PM_{2.5}$ during the follow-up was associated with 4.82 (95% CI: 0.02 – 8.54) μm per year increase in mean of the maximum CIMT. In the MESA Air study, which also had a follow-up about 2.5 years, they established an association between $PM_{2.5}$ and CCA CIMT, with 2.5 $\mu g/m^3$ higher $PM_{2.5}$ exposure during the follow-up, there was a progression of 5.0 (95% CI: 2.6 – 7.4) μm per year. (Adar, Sheppard et al. 2013). However, in our study, we did not observe a statistically significant association between $PM_{2.5}$ and CCA CIMT. Women exposed to 1 $\mu g/m^3$ higher $PM_{2.5}$ at their home address had a 0.4 (95% CI: -0.29 – 3.69) μm per year increase. Although the 95% CI interval overlapped with the MESA Air study, our point estimate was very close to null. Our study population was very young (age range: 46 – 56 years old) compared to MESA Air study (age range: 45 – 82 years old), so the sub-clinical atherosclerosis was less likely to be evident. Carotid artery thickening and plaque development are more likely to occur in segment exposed to greater turbulent blood flow and sheer stress such as the bulb and ICA. We chose out main endpoints, mean of the maximum CIMT as an outcome, which was an average of maximum value of CCA, BULB and ICA segments. Maximum CIMT can reflect a higher burden of atherosclerosis, as it is likely to

capture plaque when it is present. The maximum CIMT progression was generally higher than the mean CIMT progression, and the findings of associations between exposure and maximum CIMT progression were generally in agreement with the mean CIMT progression (Peters and Bots 2013). Also, the variation of the progressions of ICA and bulb are much larger compared to the progression of CIMT at CCA segment (Figure S 5-1). In our study, we found that a 1 $\mu g/m^3$ higher exposure to $PM_{2.5}$ during the follow-up was related to about 2.87 (95% CI: -2.54 – 8.27) μm per year higher ICA CIMT progression, and about 5.60 (95% CI: 0.49 – 12.60) μm per year higher bulb CIMT progression. The point estimates of ICA CIMT progression in response to $PM_{2.5}$ was almost comparable to the estimates with MESA Air study, and the effect size of bulb CIMT progression related to $PM_{2.5}$ was much larger.

The other two studies examining the impact of ambient $PM_{2.5}$ on progression of CIMT (Künzli, Jerrett et al. 2010, Gan, Allen et al. 2014) did not find any statistically significant relationships. These two studies also used CCA segment only for CIMT measurements, which may indicate that measuring CIMT across other part of carotid artery may be a better biomarker of atherosclerosis burden. Kunzli first reported that living within 100 m of highway or within 50 m of a major road, not $PM_{2.5}$, specifically was a predictor of CIMT progression, which might reflect a combination of pollutants as traffic is one of the major source of the pollutants in urban areas. In a more recent 10-year follow-up of MESA Air Study, no significant association was found between $PM_{2.5}$ and CCA CIMT (Kaufman, Adar et al. 2016). In this paper, the sample size was about 3500, which is who returned for exam 5 resulting in 70% of the original sample size of their 2.5-year follow-up study (Adar, Sheppard et al. 2013). The differences in sample size may reflect a selection bias. Moreover, the baseline and follow-up images were read side-by-

side, which might introduce bias and produce an artificial effect of progression (Tattersall, Gassett et al. 2014).

Furthermore, we did not observe either pollutant related to adventitial diameter. None of the other studies examining the association between air pollution and atherosclerosis used AD as a biomarker of atherosclerosis. AD is a marker of vascular remodeling and aging, which is a predictor of CVD events. In a cross-sectional study of MESA study, they found an independent association between AD and left ventricular mass (Polak, Wong et al. 2011). Among high prevalent diabetic and hypertensive population in Italy, Kozakova *et al.* found that AD was associated with prevalence of CVD events, independent from CIMT and Framingham Risk Score (Kozakova, Morizzo et al. 2017). However, we did not observe any significant association with AD related to either pollutant. As AD only measured at CCA segment, it may not reflect the atherosclerosis burden in all the carotid segments, as discussed before. Also, Saba and colleagues explored the association between AD and plaque score throughout CCA, ICA, and BULB among Japanese population, they only found moderate correlation of 0.38 (Saba, Araki et al. 2016). So, it may not be ideal to use AD instead of a direct measure, like plaque, to assess atherosclerosis burden among this young and healthy population. Within the same cohort of SWAN women at Pittsburgh site, progression of AD was related to menopause status and sex hormone level during a follow-up time upto 9 years (El Khoudary, Wildman et al. 2012, El Khoudary, Wildman et al. 2013).

In the extended models, we further adjusted for SBP, CRP, tPA and PAI-1, and the effect size did not change appreciably. These results were consistent with the findings from the MESA Air study (Adar, Sheppard et al. 2013). It has been proposed that the potential mechanism of PM causing CVD can act through three possible biological pathways (Franklin, Brook et al. 2015).

One of them is that exposure to PM can amplify systemic inflammation and oxidative stress response. However, we did not observe the effect of these biomarkers. We need to keep in mind that further adjusting for potential mediators is not an ideal method for mediation analysis. Moreover, these biomarkers were collected at the time of the outcomes; and thus, we cannot capture the temporal effect of the mediators. Our study is observational; thus, it is not an ideal way to test the mechanism of the biological pathway of the effect of PM on atherosclerosis.

To the best of our knowledge, only one paper in a community cohort in Canada used plaque as an endpoint to explore the association between air pollutants and atherosclerosis. Gan et al. reported the association between air pollution ($PM_{2.5}$ and traffic proximity) and plaque area, plaque number and total area progression (Gan, Allen et al. 2014). They found that among Chinese in Canada, people who lived within 150 m to highway or within 50 m to a major road had an increased plaque area (1.12 mm^2, CI: 0.21-2.03), but not among other racial groups or in the overall sample; and no statistically significant association was found with $PM_{2.5}$. However, this study was conducted in Vancouver, Canada, where the air pollution level is much lower than in our study area. The concentration of $PM_{2.5}$ was about 4.1 $\mu g/m^3$, which is very low compared to the levels found in US cities (average of 12-14 $\mu g/m^3$ for this time period) resulting in a very low contrast among the participants. In our study, plaque was not able to be quantified by area. However, we measured plaque index as a plaque severity, which is a semi-quantitative measure of the atherosclerosis burden. It may be a better way to look at plaque progression in this fashion. While considering plaque presence as an outcome only, we cannot determine if participants with plaque at both visits were actually progressing in plaque severity or not. For instance, participants who had plaque at both visits, but a smaller plaque index at SWAN Heart follow-up than SWAN Heart baseline were also considered as reference rather than as an outcome. They

were probably at a lower risk than who remained at same plaque index level or who had severer plaque index at follow-up. Among these participants, we found that women exposed to higher levels of $PM_{2.5}$ were more likely to have a higher plaque index at follow-up in single, as well as in two-pollutant, models after adjusting for SES factors. We did not observe any significant associations between either pollutant and plaque presence progression.

However, in our analysis, the association between O_3 and CIMT or plaque progression was not statistically significant. In a recently published abstract using the same ten-year follow-up data of MESA Air study, they found that with 3 ppb higher long-term exposure to O_3, there was a 5.6 μm (95%: 1.4 – 9.7) per 10 year increase in CCA CIMT (Wang, Sheppard et al. 2016). Translating this into 1 ppb O_3 exposure to one year CCA CIMT progression, the effect size is only 0.19 μm/year. This effect size is very small, and may not reflect a clinical meaningful progression of atherosclerosis. They also reported that a 3 ppb higher long-term exposure to O_3, there was a 20% (95% CI: 10% - 40%) higher chance to develop carotid plaque by plaque score, which is a similar concept to plaque index used in our study (Wang, Sheppard et al. 2016). The association between O_3 exposure and plaque progression were null in our findings. Since the prevalence of plaque in our population was very low, 15%, we may not have had enough power to detect an association between O_3 and plaque progression. Also, our follow-up time is much shorter, and the effect of O_3 may require a longer time to affect the CIMT. Since the production of O_3 required ultraviolet (U.S. Environmental Protection Agency 2015), which is higher in the warm season, and lower in the cold season, it can vary largely day to day. The damage from short-term episodes of high level O_3 exposure are reversible (Allen 2002). So, to observe the cumulative effect of O_3 may require continuous exposure to high-level of O_3. A retrospective study among college students, most of which were from California, found that exposure to O_3 at

childhood predicted a higher CIMT level in their college year, not the years immediately preceding their college (Breton, Wang et al. 2012). A longer follow-up may be required in the SWAN study to observe an association between O_3 and progression of CIMT.

One of the limitations of our study is that we used a less refined estimate of air pollution levels compared to other studies which used spatial-temporal modeling. However, the spatial-temporal modeling creates a more comprehensive exposure metrics of the study area, while monitors scatter in the populated or polluted areas. However, one of the cross-sectional papers from MESA Air study demonstrated that nearest monitors may present a higher contrast among the study population; and thus, possibly a stronger association between $PM_{2.5}$ and CIMT (Sun, Kaufman et al. 2013). The nearest monitor in MESA Air study used the study monitors which measured 2-week $PM_{2.5}$ level as a whole year estimation. Although we did not have hundreds of monitor sites, we were able to use the continuous monitoring data from EPA monitors throughout the whole study period. Therefore, we have few modeling and uncertainty of our exposure assessment. Also, we only have the information of the residential address and no other information of their day-to-day activities. A monitor, which is near the home, can reflect the air pollutant level of the residential address, as well as the area where the participant may take activities in. Although our study found a significant association between $PM_{2.5}$ and subclinical atherosclerosis among the women at a special stage in their life, we need to keep in mind that these findings were in a rather small sample size (about 400 women). Further studies with a larger sample size would have a stronger impact. The follow-up of our study was rather short, about 2.2 years. Considering that atherosclerosis is a life-long process (Künzli, Perez et al. 2011), a longer follow-up may be conducted in the future to confirm the association between air pollution and CIMT in this population. Also, the change of CIMT over 2 years was very small,

and the measurement via B-mode ultrasound was difficult. Lorenz et al. noted that the duration of the follow-up may affect the progression estimation and precision (Lorenz, Polak et al. 2012).

The biggest strength of this paper is *that it is the first study conducted among women* transitioning through menopause, a stage in women's life when CVD risk accelerates. Another strength in our study is the ability to measure atherosclerosis burden by CIMT more precisely. Most of the studies implemented an air exposure component into the existing cohorts to explore the association between air pollution and atherosclerosis. However, most of these multisite studies collected the CIMT measures at CCA segment only. Additionally, we were able to measure CIMT at the ICA and bulb which may be a better indicator of atherosclerosis burden, and even a better recommended biomarker maximum CIMT (Peters and Bots 2013). The other outcome included in this study was plaque index, a semi-quantitative measure of burden of plaque, which is a validated biomarker of atherosclerosis (Lammeren, F et al. 2011).

5.6 CONCLUSION

In conclusion, our study found that $PM_{2.5}$ independently contributed to subclinical atherosclerosis progression among women transitioning through menopause. These findings add to existing evidence that policies to reduce air pollution levels may be required to protect against atherosclerosis, especially in those who are already at higher risk for atherosclerosis.

5.7 ACKNOWLEDGEMENT

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5.8 TABLES AND FIGURES

Table 5-1 Baseline Characteristics by baseline $PM_{2.5}$ exposure quartiles

	$PM_{2.5}$ ($\mu g/m^3$)				p-trend
	13.16 – 15.74 (n=105)	15.74 – 16.45 (n=104)	16.45 – 17.11 (n=103)	17.11 – 23.04 (n=105)	
O_3 (ppb)	32.7 (2.7)	32.8 (2.5)	30.3 (2.8)	32.0 (2.5)	<0.001
Age (years)	51.0 (2.7)	50.2 (2.8)	50.5 (2.8)	50.6 (2.7)	0.484
White (%)	78 (74.3)	58 (55.8)	59 (57.3)	62 (59.1)	0.038
Education (%)					0.245
≤ High School	22 (21.0)	25 (24.0)	8 (7.8)	12 (11.4)	
Some College/College	27 (25.7)	31 (29.8)	35 (34.0)	39 (37.1)	
> College	56 (53.3)	48 (46.2)	60 (58.3)	54 (52.4)	
Pay for Basics (%)					0.613
Very Hard	3 (2.9)	6 (5.8)	3 (2.9)	6 (5.7)	
Some what	26 (24.8)	29 (27.9)	32 (31.1)	26 (24.8)	
Not very	76 (72.4)	69 (66.3)	68 (66.0)	73 (69.5)	
Menopausal Status (%)					0.481
Pre- and early peri-	7 (6.7)	10 (9.7)	7 (6.8)	5 (4.8)	
Late Peri-menopause	54 (51.4)	55 (53.4)	66 (64.1)	60 (57.1)	
Post-menopause	29 (27.6)	30 (29.1)	25 (24.3)	29 (27.6)	
Other	15 (14.3)	8 (7.8)	5 (4.9)	11 (10.5)	
Current Smokers (%)	13 (12.4)	17 (16.4)	11 (10.7)	15 (14.4)	0.928
BMI	28.2 (6.3)	30.1 (6.1)	29.7 (6.8)	29.1 (6.0)	0.400
SBP (mmHg)	115.4 (17.3)	120.0 (19.2)	118.4 (14.6)	120.6 (17.4)	0.063
DBP (mmHg)	73.1 (10.4)	76.2 (10.3)	76.1 (9.0)	76.0 (10.3)	0.063
Hypertension Medication (%)	16 (15.2)	37 (35.6)	21 (20.3)	30 (28.6)	0.186
Hypertension (%)	26 (25.7)	41 (40.2)	34 (34.0)	36 (34.6)	
Cholesterol (mg/dL)	203.6 (34.0)	207.0 (39.6)	194.6 (32.4)	204.5 (42.7)	0.569
LDL (mg/dL)	120.2 (29.7)	126.9 (35.0)	114.1 (27.3)	123.7 (38.7)	0.883
HDL (mg/dL)	60.1 (14.4)	56.9 (13.9)	56.9 (14.7)	56.4 (13.0)	0.065
Triglycerides (mg/dL)	97.0 (77.0, 136.0)	98.8 (75.0, 136.0)	100.5 (73.0, 140.0)	104.0 (79.0, 138.0)	0.362
Lipid-Lowering Medication (%)	3 (2.9)	12 (11.5)	11 (10.7)	5 (4.8)	0.673
Glucose (mg/dL)	87.0 (81.0, 93.0)	88.0 (82.0, 97.0)	90.0 (84.0, 96.0)	86.5 (82.0, 94.0)	0.279
Insulin (uIU/mL)	8.2 (6.6, 12.1)	9.3 (6.9, 13.4)	10.1 (7.4, 14.9)	10.0 (7.3, 15.1)	0.017
Diabetes (%)	5 (4.8)	2 (1.9)	1 (1.0)	2 (1.9)	0.164
Diabetic medication (%)	1 (1.0)	0 (0)	0 (0)	1 (1.0)	0.999

Table 5-1 continued

CRP (mg/L)	1.5 (0.5, 4.1)	1.7 (0.8, 6.0)	2.5 (0.8, 5.6)	2.3 (1.1, 5.5)	0.015
tPA (ng/dL)	6.5 (4.7, 9.0)	6.9 (5.4, 9.4)	7.2 (5.9, 10.0)	7.3 (5.3, 9.7)	0.159
PAI-1 (ng/dL)	13.9 (6.8, 23.2)	10.0 (6.4, 24.4)	16.0 (8.0, 26.9)	15.6 (9.8, 26.1)	0.121
Family History (%)	73 (72.3)	73 (73.7)	61 (61.0)	66 (70.2)	0.348
Mean CIMT (μm)	677.3 (90.9)	697.8 (101.9)	662.9 (95.9)	672.7 (80.7)	0.236
CCA CIMT (μm)	668.2 (76.9)	696.1 (104.9)	672.4 (95.7)	679.1 (90.8)	0.820
ICA CIMT (μm)	603.0 (132.4)	611.8 (133.3)	577.3 (134.7)	595.3 (129.0)	0.325
BULB CIMT (μm)	772.0 (181.5)	783.2 (185.2)	725.6 (154.4)	735.8 (135.9)	0.023
Maximum CIMT (μm)	873.9 (135.1)	896.8 (131.8)	867.0 (130.1)	860.7 (106.6)	0.217
AD (μm)	6658.8 (641.9)	6701.6 (646.2)	6778.2 (560.4)	6755.2 (604.7)	0.182
Plaque (%)	14 (13.3)	22 (21.2)	14 (13.6)	15 (14.3)	0.771
Plaque index (%)					0.783
0	91 (86.7)	82 (78.9)	89 (86.4)	88 (85.4)	
1-2	11 (10.5)	20 (19.2)	11 (10.7)	14 (13.6)	
>2	3 (2.9)	2 (1.9)	3 (2.9)	1 (0.9)	

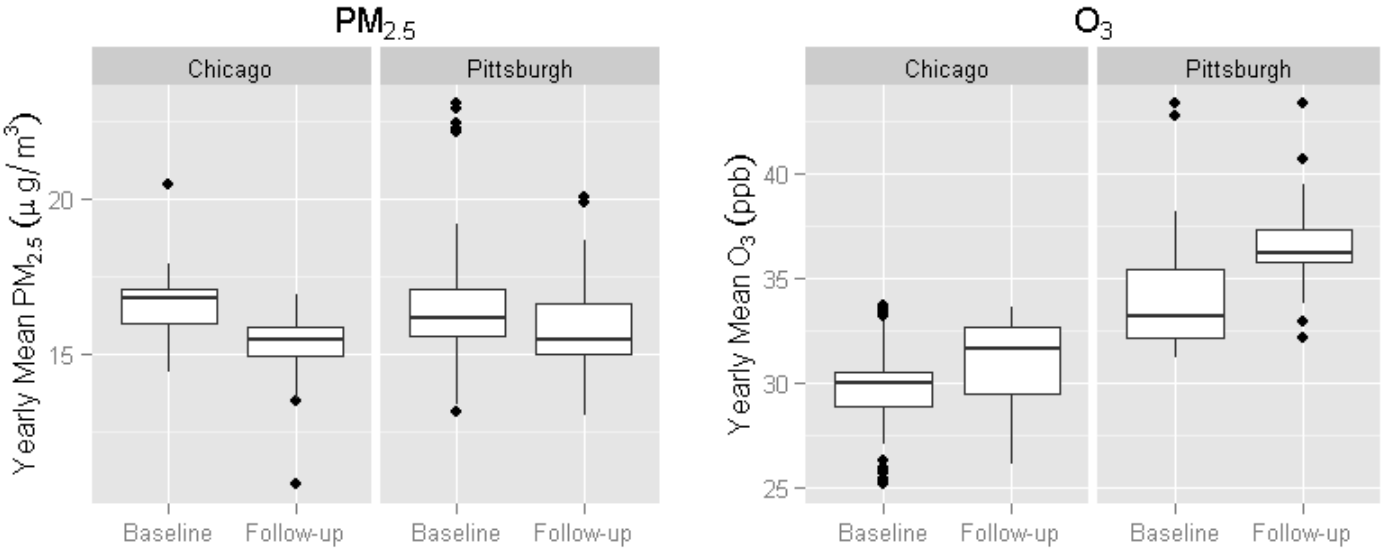

Figure 5-1 Distribution of $PM_{2.5}$ and O_3 at baseline and follow-up by two sites, Chicago and Pittsburgh

Table 5-2 Association between $PM_{2.5}$ (1 μg/m^3) and O_3 (1 ppb) and progression of subclinical atherosclerosis

	Mean CIMT (μm/yr)	Mean of Max. CIMT (μm/yr)	Adventitial diameter (μm/yr)
$PM_{2.5}$			
Model 1	1.94 (-0.82, 4.71)	3.77 (-0.36, 7.90)	-6.02 (-17.98, 5.94)
Model 2a	1.86 (-0.89, 4.61)	3.64 (-0.48, 7.75)	-6.20 (-18.15, 5.74)
Model 2b	2.25 (-0.61, 5.12)	4.28 (0.02, 8.54)	-6.83 (-19.35, 5.68)
Model 3	2.17 (-0.73, 5.08)	4.06 (-0.25, 8.37)	-5.41 (-17.93, 7.11)
O_3			
Model 1	0.23 (-0.82, 1.27)	0.31 (-1.24, 1.86)	-0.11 (-4.71, 4.49)
Model 2a	0.24 (-0.80, 1.29)	0.34 (-1.21, 1.88)	-0.13 (-4.73, 4.47)
Model 2b	0.22 (-0.90, 1.33)	0.07 (-1.57, 1.72)	-0.16 (-5.06, 4.74)
Model 3	0.32 (-0.88, 1.51)	0.05 (-1.71, 1.82)	-0.47 (-5.77, 4.82)

Notes: CIMT: carotid intima-media thickness

model 1 is the unadjusted model, only adjust for measurement related variables: site, and tech; model 2a is adjusted for socioeconomic and demographic characteristics: age, race, education, and how hard to pay the basics; model 2b is fully adjusted model, further adjusted for risk factors of cardiovascular disease, and it is the full model: BMI, smoking, triglyceride, LDL, HDL, lipids lower medication, hypertension mediation, menopause status, hormone replacement therapy, diabetes and family history of CVD or stroke; model 3 is the extended model, additionally adjusted for potential mediators: systolic blood pressure, CRP, tPA and PAI-1

Table 5-3 Association between PM2.5 (1 μg/m3) and O3 (1 ppb) and progression of per segment CIMT

	Common CIMT (μm/yr)	Internal CIMT (μm/yr)	Bulb CIMT (μm/yr)
$PM_{2.5}$			
Model 1	0.25 (-2.94, 3.44)	2.82 (-2.34, 7.99)	5.90 (0.06, 11.74)
Model 2a	0.19 (-2.99, 3.37)	2.80 (-2.37, 7.97)	5.60 (-0.23, 11.43)
Model 2b	0.40 (-2.90, 3.69)	2.87 (-2.54, 8.27)	6.54 (0.49, 12.60)
Model 3	0.54 (-2.81, 3.88)	2.48 (-3.03, 8.00)	6.19 (0.13, 12.24)
O_3			
Model 1	-0.18 (-1.40, 1.04)	0.41 (-1.54, 2.37)	1.11 (-1.70, 2.65)
Model 2a	-0.18 (-1.39, 1.04)	0.42 (-1.53, 2.38)	0.48 (-1.69, 2.65)
Model 2b	0.04 (-1.26, 1.34)	0.39 (-1.71, 2.48)	-0.07 (-2.38, 2.24)
Model 3	0.10 (-1.29, 1.49)	0.29 (-1.96, 2.54)	0.20 (-2.29, 2.69)

Notes: CIMT: carotid intima-media thickness

model 1 is the unadjusted model, only adjust for measurement related variables: site, and tech; model 2a is adjusted for socioeconomic and demographic characteristics: age, race, education, and how hard to pay the basics; model 2b is fully adjusted model, further adjusted for risk factors of cardiovascular disease, and it is the full model: BMI, smoking, triglyceride, LDL, HDL, lipids lower medication, hypertension mediation, menopause status, hormone replacement therapy, diabetes and family history of CVD or stroke; model 3 is the extended model, additionally adjusted for potential mediators: systolic blood pressure, CRP, tPA and PAI-1

Table 5-4 Association between Exposure of $PM_{2.5}$ (1 μg/m^3) and O_3 (1 ppb) and plaque presence and plaque index progression (N=319)

	Plaque Presence Progression[1]	*Plaque Index Progression*[2]
$PM_{2.5}$		
Model 1	1.21 (0.98, 1.51)	1.31 (1.04, 1.65)
Model 2a	1.21 (0.97, 1.50)	1.29 (1.02, 1.64)
Model 2b	1.16 (0.92, 1.46)	1.22 (0.95, 1.56)
Model 3	1.14 (0.89, 1.44)	1.18 (0.92, 1.53)
O_3		
Model 1	1.00 (0.98, 1.01)	0.99 (0.98, 1.01)
Model 2a	1.00 (0.98, 1.01)	1.00 (0.98, 1.01)
Model 2b	1.00 (0.98, 1.01)	0.99 (0.97, 1.01)
Model 3	1.00 (0.98, 1.01)	0.99 (0.97, 1.01)

Note: 1 reference group: participants remained plaque free, or participants who had plaque at baseline but not plaque at follow-up (N=245); 2 reference group: participants remained plaque free, or participants whose plaque index decrease at follow-up (N=252)

model 1 is the unadjusted model, only control for site; model 2a is adjusted for the SES/demographic factors, which includes age, race, education, how hard to pay for basics; model 2b is further adjusted for the cardiovascular disease risk factors, which includes BMI, smoking, cholesterol, low-density lipoprotein, high density lipoprotein, blood glucose level, and menopause status; model 3 is additionally adjusted for potential mediators, which includes SBP, CRP, tPA and PAI-1.

Table 5-5 Association between Exposure of $PM_{2.5}$ (1 μg/m^3) and O_3 (1 ppb) and plaque presence and plaque index progression in two-pollutant model (N=319)

		Plaque Presence Progression[1]	*Plaque Index Progression*[2]
Model 1	$PM_{2.5}$	1.22 (0.98, 1.51)	1.31 (1.04, 1.66)
	O_3	1.00 (0.98, 1.01)	0.99 (0.98, 1.01)
Model 2a	$PM_{2.5}$	1.21 (0.97, 1.50)	1.30 (1.03, 1.65)
	O_3	1.00 (0.98, 1.01)	0.99 (0.98, 1.01)
Model 2b	$PM_{2.5}$	1.18 (0.93, 1.50)	1.25 (0.97, 1.61)
	O_3	0.99 (0.97, 1.01)	0.99 (0.97, 1.01)
Model 3	$PM_{2.5}$	1.15 (0.91, 1.47)	1.22 (0.94, 1.58)
	O_3	0.99 (0.97, 1.01)	0.99 (0.97, 1.01)

Note: 1 reference group: participants remained plaque free, or participants who had plaque at baseline but not plaque at follow-up (N=245); 2 reference group: participants remained plaque free, or participants whose plaque index decrease at follow-up (N=252)

model 1 is the unadjusted model, only control for site; model 2a is adjusted for the SES/demographic factors, which includes age, race, education, how hard to pay for basics; model 2b is further adjusted for the cardiovascular disease risk factors, which includes BMI, smoking, cholesterol, low-density lipoprotein, high density lipoprotein, blood glucose level, and menopause status; model 3 is additionally adjusted for potential mediators, which includes SBP, CRP, tPA and PAI-1.

Table S 5-1 SWAN Heart visits corresponding to SWAN visits

SWAN Visit	3	4	5	6	7	8	9
SWAN Heart Baseline							
SWAN Heart Follow-up							
Air Exposure Collection							

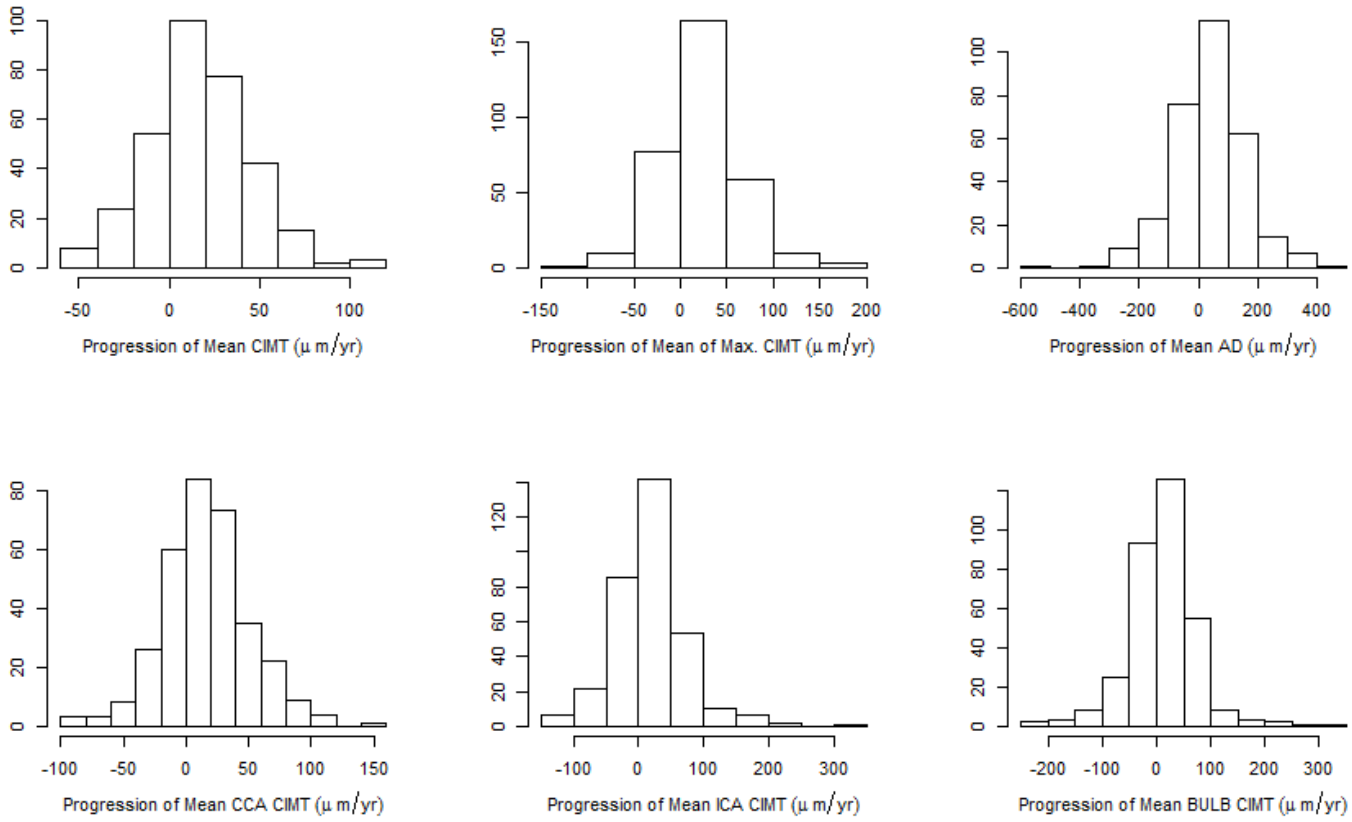

Figure S 5-1 Progression of Carotid Measures per year

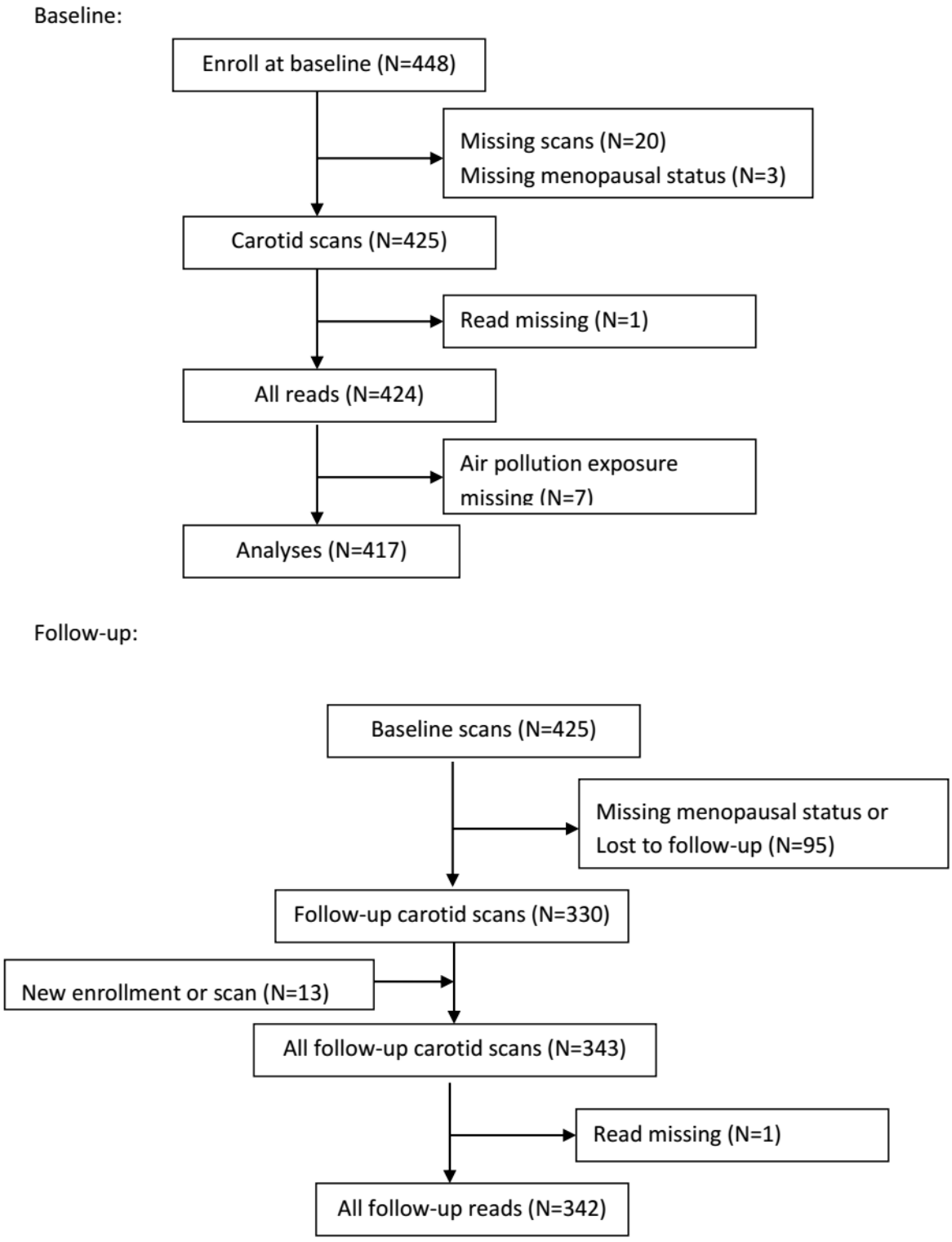

Figure S 5-2 Population included in the baseline and follow-up in the analysis

Table S 5-2 Correlation between main exposure and outcomes at baseline

	Mean CIMT	*Mean of Max. CIMT*	*IAD*	*Yearly mean $PM_{2.5}$*	*Yearly mean O_3*
Mean CIMT	1.00	0.94	0.31	-0.04	0.18
Mean of Max. CIMT	--	1.00	0.31	-0.04	0.08
AD	--	--	1.00	0.07	-0.09
Yearly mean $PM_{2.5}$	--	--	--	1.00	-0.16
Yearly mean O_3	--	--	--	--	1.00

Table S 5-3 Correlation between main exposure and outcomes at follow-up

	Mean CIMT	*Mean of Max. CIMT*	*IAD*	*Yearly mean $PM_{2.5}$*	*Yearly mean O_3*
Mean CIMT	1.00	0.92	0.37	0.02	0.11
Mean of Max. CIMT	--	1.00	0.35	0.04	0.04
AD	--	--	1.00	0.09	-0.13
Yearly mean $PM_{2.5}$	--	--	--	1.00	0.13
Yearly mean O_3	--	--	--	--	1.00

Table S 5-4 Association between $PM_{2.5}$ (1 μg/m^3) and progression of subclinical atherosclerosis after adjusting for O_3

	Mean CIMT (μm/yr)	*Mean of Max. CIMT (μm/yr)*	*Common CIMT (μm/yr)*	*Internal CIMT (μm/yr)*	*Bulb CIMT (μm/yr)*	*Adventitial diameter (μm/yr)*
PM2.5						
Model 1	1.56 (-1.28, 4.40)	2.84 (-1.37, 7.05)	0.23 (-3.07, 3.53)	2.09 (-3.19, 7.36)	5.20 (-0.78, 11.17)	-6.25 (-18.45, 5.95)
Model 2a	1.48 (-1.35, 4.31)	2.72 (-1.47, 6.91)	0.16 (-3.13, 3.44)	2.07 (-3.20, 7.35)	4.87 (-1.09, 10.83)	-6.43 (-18.62, 5.76)
Model 2b	1.92 (-1.03, 4.87)	3.40 (-0.95. 7.75)	0.43 (-2.99, 3.84)	2.25 (-3.27, 7.77)	5.82 (-0.38, 12.03)	-7.01 (-19.80, 5.79)
Model 3	1.79 (-1.61, 4.75)	3.13 (-1.22, 7.47)	0.34 (-3.10, 3.78)	2.29 (-3.29, 7.87)	5.42 (-0.70, 11.54)	-7.04 (-19.80, 5.71)

Notes: CIMT: carotid intima-media thickness

model 1 is the unadjusted model, only adjust for measurement related variables: site, and tech; model 2a is adjusted for socioeconomic and demographic characteristics: age, race, education, and how hard to pay the basics; model 2b is fully adjusted model, further adjusted for risk factors of cardiovascular disease, and it is the full model: BMI, smoking, triglyceride, LDL, HDL, lipids lower medication, hypertension mediation, menopause status, hormone replacement therapy, diabetes and family history of CVD or stroke; model 3 is the extended model, additionally adjusted for potential mediators: systolic blood pressure, CRP, tPA and PAI-1

6.0 SUMMARY

Cardiovascular disease (CVD) is the leading cause of death in the U.S. and worldwide. Despite the tremendous efforts in reducing the modifiable risk factors of cardiovascular disease and subsequent decline in CVD mortality, the overall burden of CVD morbidity and mortality is still very high. One potential risk factor for CVD is ambient air pollution, which has not yet been well studied. This dissertation examined the effect of both short- and long-term exposure to air pollution on CVD, with both clinical and subclinical outcomes.

The first manuscript used a case-crossover study design and investigated the short-term effects of multi-pollutants and CVD emergency room visits in Allegheny County, PA in 1999 – 2011. There were 181,789 cases identified over this 13-year period. Overall, O_3 on lag Day 2 was associated with the AMI emergency room visits, such that per IQR increase of O_3 exposure (25.52 ppb), was associated with a 6.6% (95% CI: 0.8% - 12.7%) increase in the odds of an AMI emergency room visit. Among women, $PM_{2.5}$ was associated with IHD, AMI and PVD. Among Blacks, $PM_{2.5}$ was related to IHD and AMI. There was a suggestive association between $PM_{2.5}$ and NO_2 and PVD in the whole population. In the early period, the association between NO_2 and O_3 and IHD and AMI were observed at lag 0; however, in the late period, when the pollution level was low, this association was observed at lag 2 – 3. There is an acute effect of $PM_{2.5}$ and O_3 on CVD emergency room visits, and this association persists in the late-period with lower exposure levels.

The second manuscript examined the association between chronic long-term exposure to $PM_{2.5}$ and O_3 and atherosclerosis using common carotid artery intima-media thickness (CCA IMT) and plaque as biomarkers of subclinical atherosclerosis. This longitudinal study was conducted among 1188 women of the Study of Women's Health Across the Nation (SWAN) from five sites with available data on both air pollutants exposure and CIMT and plaque. At the time of the carotid scan, women were on average 59.6 (±2.7) years old and a majority were postmenopausal (88.4%). The women were White (48.4%), Black (31.2%), Chinese (13.3%) and Hispanic (7.1%). We found that a 1 $\mu g/m^3$ higher cumulative exposure to $PM_{2.5}$ over 5 years was associated with an 8.0 μm (95% CI: 1.0 – 15.1) greater maximum CCA IMT after approximately seven years of exposure. This association was no longer significant after adjusting for SBP, but was not attenuated by adjusting for inflammatory biomarkers. $PM_{2.5}$ was related to mean inter-adventitial diameter and mean CCA IMT when adjusting for site and socioeconomic factors, respectively, but not after adjusting for other CVD risk factors. O_3 levels were not associated with any of the outcomes. No association was found between either pollutant and plaque presence or plaque index. $PM_{2.5}$ has a chronic long-term effect on subclinical atherosclerosis as measured by CCA IMT, which may be potentially be through a SBP pathway.

The third manuscript investigated the association between long-term air pollution and progression of subclinical atherosclerosis over 2 years among women transitioning through menopause from the Study of Women's Health Across the Nation (SWAN). This study included 417 (257 White and 160 Black) women from two sites, Chicago and Pittsburgh, with mean age of 51 years (SD = 2.8) at baseline, and most of them were peri-menopausal (60%). In the primary analysis, a 1 $\mu g/m^3$ higher yearly mean exposure to $PM_{2.5}$ during the follow-up was associated with a 4.28 (95% CI: 0.02 – 8.54) μm per year increase of mean of maximum CIMT progression,

after adjusting for confounders. The effect size did not change substantially after adjusting for the potential mediators: systolic blood pressure, C-reactive protein, tissue-type plasminogen activator antigen, and plasma plasminogen activator inhibitor 1. No association was found between O_3 and progression of CIMT or plaque. A 1 $\mu g/m^3$ higher yearly mean exposure to $PM_{2.5}$ during the follow-up contributed to about 30% (95% CI: 3% - 65%) chance of increase in plaque index progression in both the single and two-pollutant models adjusting for socioeconomic factors, but not in the fully adjusted model.

These findings suggested there is a deleterious effect of ambient air pollution on cardiovascular health from both short- and long-term exposure. There are acute effects of $PM_{2.5}$ and some other gaseous criteria pollutants on the CVD emergency room visits. We also observed the association between the chronic long-term exposure to $PM_{2.5}$ and subclinical atherosclerosis condition and progression among the mid-life women.

7.0 PUBLIC HEALTH SIGNIFICANCE

The findings from this dissertation suggest that there are possible deleterious effects of air pollution on CVD, from the perspectives of both short- and long-term exposures. These findings add evidence to support the need to reduce air pollution in the atmosphere, for both short- and long-term requirement. Currently, U.S. EPA standard for $PM_{2.5}$ is set at 12 $\mu g/m^3$, and for WHO the recommendation is set at 10 $\mu g/m^3$. However, a recently published study in the northeast of the U.S. suggested that even in areas with exposure level lower than 10 $\mu g/m^3$, there is an effect of $PM_{2.5}$ on CVD mortality. From this study, there is a possible "background" concentration of $PM_{2.5}$ at 6 $\mu g/m^3$; thus, the measures to reduce the levels are still needed. As ambient air pollution levels are determined by multiple factors, e.g. emission, meteorological factors, the intervention should be at the policy level. In the study areas, there are still days of air pollutants exceeding these standards or recommendations, indicating that there is still a need for further implementation of measures to protect heart health.

The findings in our study suggest that elevated short-term air pollution level, i.e. high level of air pollution in a single day, will increase the risk of CVD emergency room visits. If the emission level of air pollutants is constant from day to day, the high levels on certain days result from meteorological factors. Our findings may inform public health policy by providing further evidence for recommendations that susceptible populations avoid going out during these days, or avoid going out during the time of the day the pollution levels are high.

Findings from our study also imply that there is a deleterious effect of long-term exposure to air pollution on subclinical atherosclerosis, before the manifestation of clinical CVD. Each 1 $\mu g/m^3$ increase of $PM_{2.5}$ may significantly contribute to vascular change, such as intimal-medial thickness, that are indication of increasing CVD risk. Our study focused on mid-life women, during a time of increasing CVD susceptibility, thus, these findings may not be generalized to men or younger populations. In the Allegheny County, PA, there has been a long-term decrease in $PM_{2.5}$ at a rate of 0.35 $\mu g/m^3$ per year. The reduction of air pollution levels has taken a lot of time and effort. However, this may be the most cost-effective policies in the U.S. as reported by the Office of Management and Budget with about a $21 billion cost yielding up to $500 billion benefits, with most of the benefit for health outcomes. These findings suggest that the compliance of the current standards and further reducing the level even lower can contribute to improving heart health.

In conclusion, our findings support the policies and regulations in maintaining the lower air pollutant levels in the U.S., and encourage taking further measures in reducing the air pollution level even lower.

APPENDIX A: OTHER POLLUTANTS AND ATHEROSCLEROSIS

A.1 PM2.5 COMPONENTS AND ATHEROSCLEROSIS

As discussed earlier, $PM_{2.5}$ is not a single pollutant, but a very complex mixture. In recent years, the researchers have been interested in exploring which component of $PM_{2.5}$ might contribute most significantly to health outcomes. There have been an increased number of studies of $PM_{2.5}$ components carried out. This may explain some heterogeneous findings between total mass and CIMT, as PM2.5 components varied largely by the region and season (Bell, Ebisu et al. 2008, Jimenez, Canagaratna et al. 2009).

Two MESA cross-sectional studies explored the relationship between several $PM_{2.5}$ components and CIMT (Sun, Kaufman et al. 2013, Kim, Sheppard et al. 2014). However, the results of both analyses did not agree with each other. Since both papers were trying to estimate the pollution levels by different methods, the accuracy of exposure level may vary largely. Sun et al's analyses were primarily based on the monitor data with very limited modelling from other factors, e.g. emissions from traffic, meteorological factors, etc. However, the estimations of the three methods in their paper are in the same direction and significant for EC, OC and sulfur, but different effect size. The estimation using nearest monitor revealed a higher effect size than inverse distance weighting (IDW), and both are higher than the estimation using city-wide average. The monitor itself is not a source of air pollutants. IDW can address the uncertainty of

the monitor for the exposure, but cannot address the real exposure level of the certain participants by the distance from monitor. Estimating the exposure level by weighting the distance from the monitor, the value is surely less than the monitor value; and thus, result in smaller contrast of the comparison. While the city-wide average was average the pollution level of the whole city, and contrast of the exposure was much less among the participants. Kim et al. used more advanced modeling methods to estimate the exposure. With the restriction from the available monitor data and species data, the sample size in this analysis was smaller than the other study. The spatial temporal model estimated the 2-week monitor data to extrapolate the exposure of the whole study period based on the monitors from MESA Air study, while the national spatial model using the kriging technique based on the monitors from the Interagency Monitoring of Protected Visual Environments (IMPROVE) program. The exposure estimation from the latter method has a smaller range and mean estimates than the earlier one. These results using a spatial temporal model, revealed at significant associations between $PM_{2.5}$, sulfur, silicon and OC and CIMT; however, the significant association were no longer found in $PM_{2.5}$ in the national spatial model. There is possible evidence that some component of PM may contribute to increased atherosclerosis. This also implies that different estimation methods of air pollutants can yield very different results due to the modelling uncertainties. When judging the results from the paper, we need to keep in mind what estimation method of exposure was used and what the pros and cons of the method.

Black carbon is a key component of PM, and it is reduced the visibility in the environment largely (Roemer and van Wijnen 2001). One paper studied black carbon only and only cross-sectional association between black carbon and CIMT was found, but not with progression of CIMT(Wilker, Mittleman et al. 2013). The M-CHAT study examined black

carbon and CIMT as well as plaque area, number and total area progression (Gan, Allen et al. 2014). They did not find any significant associations, even in subgroups. However, the population in Vancouver, Canada had a very low contrast of exposure; and thus, they did not find very much of the association. But, these two are the only studies that examined the association between black carbon and CIMT progression. More evidence is needed to establish the association between $PM_{2.5}$ components and CIMT progression. Perhaps the size of the particles (i.e. $PM_{2.5}$) or some other components are more important than just one coarse estimation of black carbon.

The selected the components of these papers were mostly studied and usually contribute to a substantial proportion of PM (Ito, Mathes et al. 2011, Sarnat, Winquist et al. 2015). But, there are a lot of other compounds made up of PM in the atmosphere. So, further studies exploring other chemical components of PM need to be done.

A.2 NOISE, GASEOUS POLLUTANTS, AND MULTI-POLLUTANTS

Only one study used noise as an exposure and failed to find any significant results. Another study excluded noise as a major exposure, but included in the extended models. The editorial by Kunzli had pointed out that noise is very likely to be a risk factor of atherosclerosis and should not be excluded (Künzli 2013). Noise can affect health by its impact on blood pressure. Thus, it may be an interesting exposure contribute to atherosclerosis.

There are several gaseous pollutants included in this review. One of the major air pollutants is O_3. Brenton et al. conducted a study among college students and did not find anything significant related to $PM_{2.5}$, but an association was established with early childhood

exposure to O_3 (Breton, Wang et al. 2012). They also noted that the effect size did not change markedly when adjusted for PM, but decreased about 13% when adjusted for NO_2. This indicates that when assessing the exposure of multiple pollutants, the effect of one pollutant may be modified by some other pollutant. However, the estimation method of this study was regional rather than fine resolutions. So, it is very hard to draw a conclusion that the childhood exposure is a critical period of exposure to O_3 in CIMT progression. Another recently published abstract from MESA Air study also found that O_3 contribute to CIMT progression and plaque development after 10-year follow-up (Wang, Sheppard et al. 2016). However, it is the only study examine ozone's effect on atherosclerosis progression. O_3 is a very strong oxidizing agent, that it can produce free radicals; and then, add oxidative stress to human health, including atherosclerosis (Lobo, Patil et al. 2010). Limited evidence suggests that more studies are needed for O_3 and atherosclerosis.

Several studies included criteria gas pollutants as major exposures: NO_2, NOx and SO_2. These criteria gases had been monitored by EPA since 1980s (U.S. Environmental Protection Agency 2016), and there are ample data possible for the studies linking these pollutants to health outcomes, especially studies conducted at earlier years when there was no $PM_{2.5}$ data available. Also, these pollutants are oxidative active; thus, they can add the oxidative stress to the system (Meo and Suraya 2015). The M-CHAT study did not find any significant association, which may due to the low contrast of the exposure among the study population (Gan, Allen et al. 2014). All the other studies observed some significant association between exposure and CIMT (Rivera, Basagaña et al. 2012, Perez, Wolf et al. 2015, Su, Hwang et al. 2015), besides one in Netherland (Lenters, Uiterwaal et al. 2010). This study is among the young adults, which might have a low contrast of outcome compared to middle-age and old population. On the other hand, this study

found the association between NO_2 and SO_2 and PWV. This is the only study using PWV in addition to CIMT as an outcome. PWV is a biomarker to reflect the stiffness of blood vessels, and it has been applied in the younger population as a marker of atherosclerosis (Townsend, Wilkinson et al. 2015). All these evidences implied that NOx and SO_2 may contribute to atherosclerosis, as well. But, there is not enough evidence in the association with progression of atherosclerosis. Thus, more evidence may need to fill in the gap.

Another way to address multi-pollutants is to use traffic proximity and traffic density (U.S. Environmental Protection Agency 2016). Since most of the pollutants produced by traffic, e.g. NOx, CO, VOCs, are precursors to O_3 and PM. EPA has been addressed that people had activities (living, attending school and going to work) near roads, airport or railways are having some major health concerns (U.S. Environmental Protection Agency 2015). Thus, it also can be a risk factor to atherosclerosis. Most of studies only use traffic proximity to main roads or highway. This estimation method is very easy by considering the distance without taking traffic intensity into the account. Also, the estimation was not restricted by monitor availability of the time. But, the traffic intensity may vary largely from place to place (Perez, Wolf et al. 2015) even within same site. Also, if the estimation of traffic proximity over several years, the uncertainty of the traffic exposure can be very large. Over the time, there are a lot of policies and regulations about oil purification (U.S. Environmental Protection Agency 2016) and catalytic converter of automobiles (U.S. Environmental Protection Agency 2016) had been taken into the place. So, the emission of air pollutants had been reduced overtime. An estimation of air pollution at an earlier time by traffic can be higher than in recent years. Two studies used the traffic proximity of a very longtime (Gan, Allen et al. 2014, Armijos, Weigel et al. 2015). M-CHAT study with five-year follow-up did not find any significant results, besides in some

subgroups. Not account the exposure change overtime may reduce the contract of the exposure and yield a negative finding. The lifetime exposure among the children aged 6-14 years old to traffic proximity was significantly related to CIMT. However, given the limitation of traffic intensity and emission change overtime, it is hard to conclude that the childhood exposure is a critical time exposure contribute to CIMT. If the air pollution level from traffic was reducing overtime, the traffic emission at earlier years may reflect a higher contrast of the exposure level than in the more recent years. There are two articles take into account the traffic intensity by how many vehicles go through the roads per day (Rivera, Basagaña et al. 2012, Perez, Wolf et al. 2015). However, both of these two articles did not account the diesel fuel or gas fuel vehicles. The emission from diesel engines, especially the old ones are much higher than the emission from gasoline powered cars, and they also produced more PM (U.S. Environmental Protection Agency 2015). So, it is an important aspect of traffic intensity for air pollutant to consider. With this limitation, the study in Spain and Sweden found some linkage with traffic intensity and CIMT. All these results imply that traffic proximity and intensity related to CIMT, but better estimation can be made to establish more valid association. Perhaps combining the monitor data and traffic intensity can provide a better estimation of multi-pollutants.

APPENDIX B: STATISTICAL EQUATION OF CHAPTER 5.0

Generalized mixed effect model was used to estimate the effect of subclinical atherosclerosis progression of carotid measures of mean CIMT, mean of maximum CIMT, AD, CCA, ICA and BULT CIMT. The detailed model construction is as follow:

$$Y_{ij} = [\alpha_0 + \alpha_i] + [X_{i0}\beta_1] + [W_{ij}t_{ij}\beta_2] + \varepsilon_{ij}$$

Y_{ij}: CIMT or AD measures at each visit; i: individual; j: each visit, baseline or follow-up.

α_0: average baseline carotid measures of each biomarker (outcome)

α_i: subject-specific random intercept

X_{i0}: baseline measures of confounders (site, age, race/ethnicity, education, financial strain, BMI, smoking, triglycerides, LDL-c, and HDL-c levels, lipid lowering medication, hypertension medication, menopause status, hormone replacement therapy, diabetes and family history of CVD or stroke) and exposure of interest ($PM_{2.5}$ or O_3).

β_1: coefficients for average cross-sectional association between baseline confounders and exposure and outcome at baseline and follow-up

W_{ij}: yearly mean of the exposure to $PM_{2.5}$ or O_3 during the baseline to follow-up, and yearly mean of the exposure to potential mediators, which included SBP, CRP, tPA and PAI-1 for each individual

t_{ij}: time since baseline to follow-up in years for each individual

β_2: coefficients for interaction between exposure and potential mediators and time, which reflects the association between mean exposure during the follow-up related to progression of outcomes

ε_{ij}:error associated with outcomes Y_{ij}

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