FINE PARTICULATE MATTER AMBIENT AIR POLLUTION AND CARDIOVASCULAR DISEASE

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ABSTRACT

This dissertation sought to examine the effect of exposure to fine particulate matter ambient air pollution ($PM_{2.5}$) on cardiovascular disease and biological pathways linking them.

In the first manuscript, $PM_{2.5}$ air pollution was significantly associated with IHD and PVD mortality in Allegheny County, PA at a lag of 5 days, for the period 1999-2011. The risk of IHD mortality due to $PM_{2.5}$ was significantly greater for individuals who died outside of a hospital or nursing home compared to deaths in the hospital or nursing home.

In the second manuscript, overall, there were no appreciable effects of short and long-term exposure to $PM_{2.5}$ air pollution with regard to biomarkers of cardiovascular risk i.e. CRP, WBC count, homocysteine and fibrinogen, after adjusting for demographic and cardiovascular risk factors in adult NHANES participants for the period 2001-2008. However, we did find some evidence suggesting stronger associations of $PM_{2.5}$ with biomarkers of cardiovascular risk in participants with elements of metabolic syndrome e.g., obesity, diabetes, hypertension and smokers.

In the third manuscript, individuals with preexisting metabolic syndrome compared to individuals without preexisting metabolic syndrome, showed a stronger positive response in systemic inflammation, as manifested by CRP and WBC count, in association with $PM_{2.5}$ air

pollution (both short term and long term), after adjusting for demographic and cardiovascular risk factors in adult NHANES participants for the period 2001-2008

Further research is warranted to confirm these findings in large cohorts. With one third of the U.S. population compromised by metabolic syndrome, the health impact of particulate air pollution in this sensitive population is likely to be significant and emphasizes the public health importance of this body of work.

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1.0 INTRODUCTION

According to World Health Organization, globally, 3.7 million deaths were attributable to ambient air pollution in 2012. 80 % of these deaths attributable to ambient air pollution were due to ischemic heart diseases and stroke[1]. Cardiovascular diseases (CVD) including ischemic heart diseases and stroke is a major cause of death in United States (US) and globally. Although, rates of deaths attributable to CVD have declined over the years in US, it still accounts for 1 in every 3 deaths. The economic burden to society is huge considering that the total direct and indirect cost of CVD and stroke in the United States for 2010 is estimated to be $315.4 billion [2]. American Heart Association (AHA) in its 2010 statement reported that exposure to short term and long term levels of $PM_{2.5}$ increases risk for CVD morbidity and mortality. Many credible pathological mechanisms have been elucidated that support the biological plausibility of these findings. These include systemic inflammation, systemic oxidative stress, thrombosis and coagulation, systemic and pulmonary arterial blood pressure responses, vascular (including endothelial) dysfunction, cardiac ischemia, and heart rate variability/autonomic dysfunction[3]. However, the epidemiological evidence that explains these mechanisms is still inconclusive.

1.1 PARTICULATE MATTER AMBIENT AIR POLLUTION AND CARDIOVASCULAR DISEASE

Epidemiological studies have consistently demonstrated link between particulate matter (PM) ambient air pollution and CVD. In broad term, studies can be separated into those that have investigated the health effects of acute and chronic air pollution exposure. Brook et al. (2010) extensively reviewed available evidence to study relationship of PM air pollution and CVD. In a scientific statement from the AHA, they summarized that there is a small, yet consistent association between increased mortality and short term elevations in PM_{10} and $PM_{2.5}$. They concluded that for every 10 $\mu g/m^3$ elevation in $PM_{2.5}$ during the preceding 1 to 5 days, there is increase of 0.4% to 1.0% in daily mortality specifically cardiovascular death and for every 10 $\mu g/m^3$ elevation in long term average $PM_{2.5}$ exposure, there is approximate 10 % increase in all-cause mortality. Mortality risk from CVD was elevated to similar extent, although range was very broad. Hospital admissions due to CVD were also elevated in response to daily changes in PM levels. They also concluded that there is a strong evidence of PM effect on ischemic heart disease, moderate (yet growing) for heart failure & ischemic stroke and modest or mixed for peripheral vascular and cardiac arrhythmia/arrest[3].

1.1.1 $PM_{2.5}$ Ambient Air Pollution and Cardiovascular Mortality

Several studies have been conducted in Unites States and other parts of world after AHA statement in 2010 that examined relationship of $PM_{2.5}$ exposure with CVD mortality. In a study conducted in New York, Ito et al. (2011) found that $PM_{2.5}$ was associated with CVD mortality in the warm season at lag 0 [% Excess Risk (ER) = 2.0 %; 95% confidence interval (CI), 0.7–3.3;

per 10 μg/ m^3] and lag 1 day (1.9 %; 95% CI, 0.8–3.1) and in the cold season at lag 1 day (1.0%; 95% CI, –0.1 to 2.2). [4]. Zhou et al. (2011) found significant positive association for cumulative effect from lag 0 to 2 days for $PM_{2.5}$ for all-cause and cardiovascular mortality in the warm season in Detroit, suggesting a role of secondary pollutants; in contrast, Seattle showed positive associations in Winter [5].

Shang et al. (2013) conducted a meta-analysis of time series studies from China, 1990-2012 and found that each 10 μg/m³ increase in $PM_{2.5}$ was associated with a 0.38% (95% CI: 0.31, 0.45) increase in total mortality, a 0.51% (95% CI: 0.30, 0.73) in respiratory mortality, and a 0.44% (95% CI: 0.33, 0.54) in CVD mortality. The associations for PM_{10} were similarly strong[6]. In 2013, Shah et al. conducted a meta-analysis of global studies conducted during 1984-2012 that examined relationship of the daily levels of particulate matter and gaseous pollutants with heart failure hospitalizations or heart failure mortality. They found that increases in particulate matter concentration were associated with heart failure hospitalization or death ($PM_{2.5}$, 2.12% per 10 μg/m³, 95% CI: 1·42–2·82; PM_{10}, 1·63% per 10 μg/m³, 95% CI: 1·20–2·07). Strongest associations were seen on the day of exposure, with more persistent effects for $PM_{2.5}$[7]. Samoli et al. (2014) conducted meta-regression of time series in 10 European Mediterranean metropolitan areas. They found a statistically significant increase in cardiac deaths by 1.33% (95% CI: 0.27, 2.40%) for a 10 μg/m³ increase in six days' $PM_{2.5}$ exposure. Stronger effects were observed in the warm season. Atkinson et al. (2014) conducted a meta-analysis and found that a 10 μg/m³ increment in $PM_{2.5}$ was associated with a 1.04% (95% CI 0.52-1.56) increase in the risk of death. However, worldwide, there was substantial regional variation (0.25% to 2.08%). The associations for respiratory causes of death were larger than for cardiovascular causes, 1.51% (1.01% to 2.01%) vs 0.84% (0.41% to 1.28%)[8].

As of now, three studies have examined the relationship between daily $PM_{2.5}$ and mortality in Pittsburgh. Chock et al. (2000) conducted a time series from period 1989-1991 and found statistically significant results for PM_{10} with daily non-accidental mortality for age < 75 in 0- lag model. However, due to small signal to noise ratio, they could not credibly ascertain the relative association of $PM_{2.5}$ and mortality [9]. Franklin et al. (2007) in a multicity analysis, found statistically significant association between daily lag 1 $PM_{2.5}$ and all-cause mortality in Pittsburgh [10]. In another multicity analysis, Franklin et al. (2008) found overall statistically significant association of $PM_{2.5}$ and non-accidental deaths and CVD mortality, but, Pittsburgh specific results were not available[11].

1.2 PARTICULATE MATTER AMBIENT AIR POLLUTION AND BLOOD MARKERS OF CVD RISK

The first study to link PM air pollution with blood markers was conducted in Belfast and Edinburgh, UK in 1999. This study found that city center PM_{10} measurements over three days were positively associated with C-reactive protein (CRP) ($p<0.01$), negative correlated with fibrinogen ($p<0.05$) and not associated with white blood cell count (WBC) ($p<0.61$). However, none of the personal exposure estimates averaging over three days were associated with any of these outcomes. The variables adjusted for analysis were city, season, temperature, and repeated individual measurements. Since 1999, there have been number of epidemiological studies that attempted to explain biological mechanism linking particulate air pollution with blood markers of systemic inflammation (CRP and WBC), systemic oxidative stress (homocysteine) and thrombosis and coagulation (fibrinogen), yet the results are inconclusive. Some of the possible

reasons behind inconsistent findings are errors in exposure assessment, different exposure time period, difference in PM constituents, relative small sample size and inadequate control of confounders.

1.2.1 Particulate Matter Ambient Air Pollution and C - reactive protein

CRP is a circulating acute-phase reactant that is increased many-fold during the inflammatory response to tissue injury or infection. It is synthesized primarily in the liver and its release is stimulated by interleukin 6 (IL-6) and other pro-inflammatory cytokines.[12] CRP is linked to the development of cardiovascular diseases [13].

Out of all the biomarkers studied in relation to air pollution, CRP is the most extensively studied. Li et al. (2012) published a systematic review of effect of particulate matter air pollution on CRP, including cross-sectional, longitudinal and randomized crossover trial studies. Except one study among adults with type 1 or 2 diabetes that found significant association of 7 day mean of PM_{10} with CRP changes, they reported largely null findings from cross-sectional studied reviewed [14-21]. One possible reason for largely null findings suggested by them was intake of anti-inflammatory medications among adults. Results from three longitudinal studies conducted in occupational settings were positive suggesting high PM exposure levels inducing CRP synthesis and large variations of PM level enabling detection of meaningful changes of CRP levels [22-24]. Additionally, less likely use of medications that might affect CRP levels in a healthy occupational population. However, results were inconsistent from ten longitudinal studies conducted among the healthy general population [25-34] and adults with chronic inflammatory conditions like CVD [29, 35-38], COPD [29, 39], diabetes and obesity [30, 40-42]. Except one, all other seven randomized crossover trial reported null findings[43].

A total of seven longitudinal studies have been published after Li et al.'s systematic review. Out of the four panel studies, only Meier et al. (2014) found positive association [44-47]. Meier et al. (2014) found that a 10 μg/ m^3 increment in $PM_{2.5}$ exposure was associated with a 5.56 % (1.05-10.27) increase in CRP among 18 nonsmoking male highway maintenance workers in US [47]. Among three large cohort studies, two found significant association. Bind et al. (2012) did not found association among short and intermediate exposure (windows of 4/24 hours, and 3 to 28 days moving average preceding each subject's examination) of $PM_{2.5}$, particle number and black carbon and CRP among prospective cohort of 704 subjects from the Normative Aging Study[48]. Ostro et al. (2014) conducted analysis of SWAN cohort of 1923 mid-life women and reported that a 10 μg/m^3 increment in annual $PM_{2.5}$ exposure was associated with a 25.5% (95% CI: 10.2, 42.9) increase in CRP [49]. Hennig et al. (2014) analyzed a prospective population-based German cohort of 4,793 participants (45-75 years of age) and found that a 1 μg/m^3 increase in residential long-term total $PM_{2.5}$ was associated with a 4.53% increase in hs-CRP concentration (95% CI: 2.76, 6.33). The CRP was 17.89% (95% CI: 7.66, 29.09) and 7.96% (95% CI: 3.45, 12.67) higher in association with 1 μg/m^3 increases in residential long-term traffic- and industry specific $PM_{2.5}$, respectively[50].

Two cross-sectional studies were conducted outside US in specific populations i.e. traffic policemen and COPD patients. Zhao et al. (2013) analyzed a cross-section of 101, 25-55 years old nonsmoker traffic policemen in Shanghai, China with no cardiopulmonary disease and no current medication. They found that $PM_{2.5}$ exposure is associated with the increases in hs-CRP of 1.1% (0.6–1.5) [51]. However, Dadvand et al. (2014) did not found clear pattern of associations across lags 1–10 prior to blood sampling in 251 clinically stable COPD patients in Barcelona metropolitan areas, Spain.[52].

The relationship of PM exposure to CRP has been examined extensively. After, Li et al. (2012), seven additional longitudinal studies have been conducted. Out of three longitudinal studies of large cohort, two observed positive association of long term $PM_{2.5}$ exposure. Two panel studies of healthy volunteers reported null findings, whereas two panel studies of occupational settings reported positive association.

1.2.2 Particulate Matter Ambient Air Pollution and White Blood Cell

WBC is an indicator of cellular response to inflammation and considered a potentially useful predictor of prevalent or incident CVD, according to the joint scientific statement by the Centers for Disease Control and Prevention and the American Heart Association [13].

As of now, four panel studies have been conducted examining relationship of particulate matter air pollution and WBC count [25, 30-32, 37]. Except Dubowsky et al. (2006), none of them found significant association. Dubowsky et al. (2006) analyzed 44 senior citizens (> 60 years of age) in Missouri, USA. They found positive associations between longer moving averages of $PM_{2.5}$ and WBCs across all participants with a 5.5% (95% CI: 0.10, 11) increase per interquartile increase (5.4 $\mu g/m^3$) of $PM_{2.5}$ averaged over the previous week. Associations with WBC counts remained significantly elevated through the 14-day mean but declined with longer moving average. Additionally, WBC counts generally increased with IQR changes in ambient Black Carbon (BC) [30].

Out of five cross-sectional studies, only two studies i.e. Schwartz et al. (2001) and Chen et al. (2008) found significant positive association [14, 19, 53-55]. Schwartz et al. (2001) analyzed national sample of US population from NHANES III and found that in single-pollutant models, PM_{10} (Lag 0 and 1 before blood collection) was associated with WBC count. In two-

pollutant models, PM_{10} (Lag 0 and 1 before blood collection) remained a significant predictor of WBC count controlling for SO_2. For WBC, the OR of being in the top 10% for the same IQR change was 1.64 (95% CI; 1.17, 2.30). These results were stable with control for indoor exposures, dietary risk factors and serum cholesterol[54]. Chen et al. (2008) also conducted secondary analysis of NHANES III and found statistically significant association between WBC count and estimated long term PM_{10} levels ($p = 0.035$). Participants from the least polluted areas (1-year PM_{10} < 1st quartile cutoff: 27.8 $\mu g/m^3$) had lower WBC counts than the others (difference = 145 x 10(6)/L; 95% CI, 10-281)[55].

Except two cross-sectional studies that examined long-term PM (specifically PM_{10}) exposure, all other studies have only examined short term (up to 7 days before blood collection) PM exposure. None of the cross sectional studies have examined $PM_{2.5}$ exposure. Also, no longitudinal analysis of large cohort has been conducted. The results are inconsistent and more epidemiological studies are needed to examine this relationship, especially focusing on $PM_{2.5}$ exposure.

1.2.3 Particulate Matter Ambient Air Pollution and Fibrinogen

Fibrinogen is a circulating glycoprotein that acts at the final step in the coagulation response to vascular and tissue injury. It is synthesized in liver and cleared by thrombin to form soluble fibrin fragments, which are the most abundant component of blood clots[12].Several prospective epidemiological studies have shown independent association of fibrinogen and incident cardiovascular disease[13].

After CRP, fibrinogen is the most studied biomarkers in relation to particulate air pollution. A total of 16 longitudinal studies conducted have largely reported null findings [28,

29, 31, 33, 35, 44, 46, 56-58], however two longitudinal studies of large cohort found significant positive findings. Ruckerl et al. (2007) analyzed a prospective longitudinal cohort of 1,003 MI survivors in six European cities and found that five day cumulative exposure to PM_{10} (13.5 μg/ m^3) was associated with increased fibrinogen concentrations (percent change of arithmetic mean, 0.6; 95% CI, 0.1–1.1). In addition to the effect for the cumulative exposure, they also found significant increase for fibrinogen with lag 3 for $PM_{2.5}$ and PM_{10} [38]. Bind et al. (2012) analyzed a prospective cohort of 704 subjects from the Normative Aging Study and found that an IQR increase in exposure (3-day moving average) was associated with a 2.4% (95% CI; 0.1,4.81) increase in fibrinogen for particle number and 2.6% (95% CI; 0.9,4.3) increase for BC. In contrast, $PM_{2.5}$ was not associated with fibrinogen [48].

Chuang et al. (2007) conducted a panel study among 76 young healthy students aged 18 to 25 years from a university in Taipei and observed that 1 day average PM_{10} and 1-3 day averages sulfate were positively associated with fibrinogen. None of the other particulate matter and its constituents was associated with fibrinogen [27]. Wu et al. (2012) panel study of 40 healthy college students in Beijing, China and found significant associations with Ca, Na, Mg, Ba, Fe, Ti, Co and Cd ($p<0.05$); no association between particles (i.e. PM_{10}, $PM_{10-2.5}$, $PM_{2.5}$) and fibrinogen[44].

Hildebrandt et al. (2009) conducted a panel study among 38 male patients with chronic pulmonary disease during winter 2001/2002 in Germany and found a consistent increase in fibrinogen for lag 3 with all particulate pollutants (i.e. UFP, PM10, EC, OC) except for ACP. Additionally, the 5-day mean concentrations of UFP demonstrated a positive significant association[39]. Huttunen et al. (2012) conducted a panel study of 52 elderly ischemic heart disease patients living in the city center of Kotka, Finland and observed statistically significant

positive association with $PM_{2.5-10}$ at lag 1. There was a change of 1.15% (95% CI; 0.18, 2.12) per 5.37μg/m^3 (IQR) [37]. Rückerl et al. (2014) analyzed three panels of non-smoking individuals (1) with type 2 diabetes (2) with impaired glucose tolerance (3) with a potential genetic predisposition in Augsburg, Germany. They observed small positive associations with lag 3 for UFP for the panel with potential genetic susceptibility and small but significant positive associations for $PM_{10-2.5}$, PM_{10}, BC and CO for several lags for the panel of T2D/IGT [59].

Similar to longitudinal studies, largely null findings were reported from 9 cross-sectional studies conducted so far among randomly selected healthy adults [14, 16, 53, 60, 61]. Pekkanen et al. (2000) analyzed a cross-section of 7205 office workers in London, UK and observed significant associations of PM_{10} only in the warm season. During warm season, there was difference of 3.24% in fibrinogen level (1.29, $p<0.05$) for an increase in PM_{10} (lag 1) from 10th to 90th percentile (i.e. 33.1 μg/m^3) [62]. Schwartz et al. (2001) analyzed national sample of US population from NHANES III and found PM_{10} was associated with fibrinogen in single-pollutant models. In two-pollutant models (with NO_2); PM_{10} was also positively associated with fibrinogen. The magnitude of the effects was modest [e.g., 13 mg/dL fibrinogen for an IQR change in PM_{10}, 95% CI: 4.6, 22.1 mg/dL]. However, the OR of being in the top 10% of fibrinogen for the same IQR change was 1.77 (95% CI: 1.26, 2.49). These results were stable with control for indoor exposures, dietary risk factors and serum cholesterol [54]. Zeka et al. (2006) conducted cross-sectional analysis on 710 subjects of the VA Normative Aging Study cohort, USA and observed PN concentration in the prior 48 h was associated with increased fibrinogen levels (4.19%; 95% CI: 2.04, 6.34), as were concentrations the week before (2.14%; 95% CI: 50.05, 4.23). The relative change for 1 SD change in the concentration level to 4 week moving average BC for fibrinogen was 1.78% (95% CI: 0.19, 3.36). No association was seen

with $PM_{2.5}$ and Sulphate [19]. Hoffmann et al. (2009) analyzed baseline data from prospective cohort of 4032 participants from densely populated and highly industrialized Ruhr area, Germany. They reported that in the adjusted analysis, a cross-sectional exposure difference of 3.91 $\mu g/m^3$ in $PM_{2.5}$ (interdecile range) was associated with increases in fibrinogen of 3.9% (95% CI: 0.3, 7.7) in men, whereas no association found in women. Short-term exposures to air pollutants and temperature did not influence the results markedly [20].

Emmerechts et al. (2012) analyzed a cross section of 233 diabetic patients in Belgium and found that concentrations of fibrinogen correlated positively with PM_{10} at day-2 and day-3, as well as with the mean PM_{10} concentration over 1 week. Each 10 $\mu g/ m^3$ increase in the mean concentration of PM_{10} over the preceding week at the patient's residence elevated fibrinogen levels by 4% (95% CI: 1,7) [21]. Dadvand et al. (2014) in cross-section of 251 clinically stable COPD patients did not observe a clear pattern of associations across the lags for $PM_{2.5}$ [52].

Broadly, studies have examined short, intermediate and long term exposure. Largely null findings have been reported from panel studies of healthy young volunteers. Panel studies conducted among susceptible population i.e. cardiopulmonary patients have inconsistent results. Three studies have examined intermediate exposure (> 7 days to <12 months), however, only one longitudinal study found positive association of particle number with fibrinogen. Out of three studies that examined long term exposure (annual average), only one study observed positive association between annual average PM exposure and fibrinogen.

1.2.4 Particulate Matter Ambient Air Pollution and Homocysteine

Homocysteine is a highly reactive, sulfur-containing amino acid formed as a by-product of the metabolism of the essential amino acid methionine. Mechanistic studies have demonstrated that

homocysteine may induce vascular damage by promoting platelet activation, oxidative stress, endothelial dysfunction, hypercoagulability, vascular smooth muscle cell proliferation, and endoplasmic reticulum stress. Recent meta-analyses that included larger numbers of prospective studies and/or corrected for regression dilution bias (the intra-individual variability in homocysteine levels over follow-up) do show a significant association between homocysteine and CVDs.[12]

As of now, only four studies have been conducted to examine relationship of particulate air pollutants and homocysteine. Ren et al. (2010) conducted longitudinal analysis of 1000 white non-Hispanic older men (mean age, 72.00 +/- 7.2 yrs.) of The Normative Aging Study cohort and found that IQR increases in $PM_{2.5}$ and BC (7-day moving averages) were associated with 1.5% (95% CI; 0.2, 2.8) and 2.2% (95% CI; 0.6%, 3.9%) increases in total plasma homocysteine, respectively [63]. Wu et al. (2012) conducted a panel study of 40 healthy college students in Beijing, China and did not find significant association between cumulative average concentrations of $PM_{2.5}$ and its chemical constituents during the preceding 1 (24 hours) to 6 (144 hours) days prior to the blood collection and tHCy [44].

Bacarelli et al. (2007) conducted a cross-sectional analysis of 1,213 healthy subjects from Lombardia, Italy. Overall, they did not found any significant association. However in smokers, 24-hr PM_{10} levels were associated with 6.3% (95% CI: 1.3, 11.6) and 4.9% (95% CI: 0.5, 9.6) increases in fasting and post methionine-load tHcy, respectively, but no association was seen in nonsmokers (p-interaction = 0.005 for fasting and 0.039 for post methionine-load tHcy). For smokers, 7-day PM_{10} was associated with a non-significant 3.3% (95% CI: –1.5, 8.4) increase in fasting tHcy and a significant 5.2% (95% CI, 0.8 to 9.8; $p < 0.05$) increase in PML tHcy [64]. Park et al. (2008) analyzed a cross-section of 960 community residing elderly men from The

Normative Aging Study cohort and did not found any significant association of $PM_{2.5}$ with total homocysteine. However, statistically significant positive associations of total homocysteine were observed with traffic-related particles (black carbon and organic carbon) and this was more pronounced in persons with low concentrations of plasma folate and vitamin B_{12}. After controlling for all potential confounders, an IQR increase in concurrent day BC (0.66 mg/m3) was related to a 3.13% (95% CI, 0.76–5.55%) increase in tHcy. No association was observed with sulfate, an indicator of coal combustion particles, or $PM_{2.5}$ [65].

The epidemiological studies conducted have only examined short term (up to 7 days before blood collection) PM exposure. More studies are needed for any definite conclusion; however it seems that short term PM exposure may be associated with homocysteine, especially among smokers and older people.

1.3 PARTICULATE MATTER AMBIENT AIR POLLUTION AND INFLAMMATORY MARKERS (CRP AND WBC) IN ADULTS WITH ELEMENTS OF METABOLIC SYNDROME

Out of six longitudinal studies examining the relationship of particulate matter ambient air pollution and CRP in adults with elements of metabolic syndrome, three did not show any significant association [40-42]. Dubowsky et al. (2006) in panel study of 44 senior citizens (> 60 years of age) found that associations between $PM_{2.5}$ and CRP were consistently, and often significantly, elevated among the 8 individuals with diabetes (26 repeated samples), 14 individuals with obesity (41 repeated samples), and 4 individuals with concurrent diabetes, obesity, and hypertension (14 repeated samples). They reported that an IQR (6.1 $\mu g/m^3$) increase

in 5 day mean $PM_{2.5}$ was associated with 48% increase (95 % CI: 5.3-109) in CRP for persons with obesity, 74% increase (95% CI:18-158) for persons with diabetes, and an 81 % increase (95% CI:21-172) for persons with obesity, diabetes and hypertension compared with a 12% increase (95% CI: -25-67) for individuals without any of these conditions[30]. Rückerl et al. (2014) conducted study among three panels of non-smoking individuals (1) with type 2 diabetes (2) with impaired glucose tolerance (3) with a potential genetic predisposition in Germany. They did not found any clear pattern of association among those who had type 2 diabetes and with impaired glucose tolerance. However, for the panel with potential genetic susceptibility, a consistent and clear increase in CRP in association with all air pollutants (i.e. UFP, PM_{10}, $PM_{10-2.5}$, $PM_{2.5}$, BC and OC) for lag 0 to 4 and 5-day average exposure was observed [59]. Ostro et al. (2014) observed the strongest relation of $PM_{2.5}$ to CRP among diabetics (72.1%, 95% CI 2.9-187.8 for a 10 $\mu g/m^3$ change in $PM_{2.5}$), and associations greater than 40% were noted for the high-age group and several other subgroups such as, high blood pressure, on hormone therapy[49].

Zeka et al. (2006) in a cross-sectional analysis of 710 subjects of the VA Normative Aging Study cohort found no statistically important difference for any category of effect modifiers ((age (<78, >=78 years); BMI, use of medications i.e. anti-hypertensive and cardiac medication and hypertension), however, there was suggestion that older age (78 years or older) increased the effect of Particle Number concentrations on CRP levels. A 4-fold difference was seen for the association between BC and CRP in the presence of obesity [19]. Emmerechts et al. (2012) in a cross sectional sample of 233 diabetic patients in Belgium found significant positive correlations between PM_{10} exposure at the patient's residence and CRP concentrations for PM_{10} exposure windows within 1 week , with positive but only borderline significant values ($0.05 < P$

< 0.10) for the longer time windows up to 6 months. Each 10 μg/m^3 increase in the mean PM_{10} concentration over the preceding week at the patient's residence increased the CRP by 23% (95% CI: 5–45) [21].

Three studies have examined the relationship of particulate matter ambient air pollution and WBC count in adults with elements of metabolic syndrome. Chen et al (2008) and Emmerechts et al. (2012) found significant association whereas Dubowsky et al. (2006) did not. Chen et al. (2008) reported graded association between PM_{10} and WBC across subpopulations with increasing MS components, with 91 x 10^6/L difference in WBC for those with no MS versus 214, 338, and 461 x 10^6/L for those with 3, 4, and 5 metabolic abnormalities (trend-test p = 0.15) was also noted[55]. Emmerechts et al. (2012) observed that for each 10 μg/m^3 increase in the mean PM_{10} concentration over the preceding week at the patient's residence increased the WBC by 7% (95% CI: 2,12) [21].

1.4 EXPOSURE ASSESSMENT FOR AMBIENT AIR POLLUTION

Exposure assessment of study participants is one of the most challenging aspects of air pollution epidemiology. Over the years, methods for assessment have developed starting from proximity based to methods based on air quality networks to more advanced methods relying on personal exposure. Broadly, panel studies have relied on fixed site monitoring stations. However, recent studies have incorporated personal exposure assessment for better estimates. Individual's activity pattern and fixed site monitors were used to estimate personal exposure of participants in study by Seaton at al. (1999)[31]. Sorensen et al. (2003) utilized equipment for personal sampling that was placed in a backpack, which the subjects carried or placed nearby when they were indoors

[56]. Sullivan et al. (2007) and Delfino et al. (2008) measured exposure at participant's residence (outside of residence) or very close to their residence [29, 57].

In case of large prospective cohort and cross-sectional studies, a range of methods have been used for exposure assessment. Pekkanen et al. (2000), Steinvil et al. (2008) and Panasevich et al. (2009) mainly relied on data from fixed site monitoring network [14, 61, 62]. Liao et al. (2005) assigned exposure values equal to county specific daily average pollutant exposures, calculated for each pollutant by averaging all available monitor-specific daily averages from all operating monitors within a county on any calendar date [53]. Bacarelli et al. (2007) used information from monitors located at 53 different sites throughout Lombardia, Italy to identify nine different study areas in the region characterized by homogeneous within-area air pollution concentrations. Averaged mean hourly concentrations were used for exposure assessment after assigning each of the study subjects to one of the nine pollution areas, based on their residence [60].

Schwartz et al. (2001) and Emmerechts et al. (2012) employed geostatistical methods for modelling exposures using inverse distance weighting (IDW) and kriging respectively[21, 54]. Generally, kriging is a better method as degree of uncertainty in spatial predictions at un-sampled sites can be calculated thus indicating where interpolation is less reliable. Also, intrinsic nature of kriging model better deals with erroneous local variability, yet poor edge representation is still an issue. However, IDW methods are simpler to apply and more suitable where sampling network is sparse and errors are assumed to be large [66].

Dadvand et al. (2014) used land use regression (LUR) model to obtain spatial estimates of pollutants and temporally adjusted for assigning to participants[52]. LUR models are relatively inexpensive and provide reliable estimates of traffic related air pollution when

adequate land use, transportation and pollution monitoring data are available. The main limitation of this method is its area specificity [66].

Forbes et al. (2009) estimated annual average background exposure to PM_{10} and other pollutants for each 1 km^2 of England from an emission inventory by using air dispersion models including the effect of weather conditions [16]. Dispersion models generally rely on Gaussian plume equations and have advantage of incorporating both spatial and temporal variation of air pollution within a study area without need for dense monitoring networks. The disadvantages of this model is relatively costly data input, unrealistic dispersion pattern assumptions, need for extensive cross-validation and estimate errors die to temporal mismatches[66].

Hoffmann et al. (2009) used the EURAD (European Air Pollution Dispersion) model, a dispersion and chemistry transport model to estimate the annual mean values for background $PM_{2.5}$ concentrations on a spatial scale of 5-km grid-cell length and assigned these to the addresses of the participants. The EURAD model uses input data from official emission inventories on a scale of 1 km^2, including industrial sources, household heating, traffic, and agriculture data on hourly meteorology and regional topography. Surface concentrations were calculated by dispersing emissions in horizontal strata, taking chemical reactivity and transport processes into account. From the model output, daily surface concentrations of air pollutants for a grid-cell length of 5 km were calculated and validated by comparing the model-derived values with measured air pollution data from monitoring sites [20]. Due to their high implementation cost, data requirements and 1-km grid resolution, integrated meteorological-emission models usage is limited in air pollution epidemiological studies [66].

1.5 BIOLOGICAL MECHANISM OF PARTICULATE MATTER AMBIENT AIR POLLUTION AND CADIOVASCULAR DISEASES

Particulate matter inhalation can lead to cardiovascular disease events by three generalized intermediary pathways. These pathways include pathway 1, the pulmonary oxidative stress and inflammation leading to systemic oxidative stress and inflammation that further releases pro-inflammatory mediators (e.g. cytokines, activated immune cells, or platelets) or vasculoactive molecules (e.g. ET, possibly histamines or micro particles) from lung based cells; pathway 2, systemic ANS imbalance or heart arrhythmia by particle interaction with lung receptors or nerves; and pathways 3, translocation of PM and/or its constituents into systemic circulation[1]. These pathways represent simplified version of complicated biological processes. There is a strong experimental evidence of oxidative stress as a critically important cause and consequence of PM-mediated cardiovascular effects at the molecular level. Activation of pathway 2 and 3 within minutes and hours of PM inhalation leads to ANS imbalance (e.g. elevated BP, arrhythmias, and increased platelet aggregation), along with direct effects of circulating PM constituents (e.g. vasoconstriction, elevated BP, possibly platelet aggregation). These effects would be clinically meaningful in those who are susceptible in terms of vulnerable plaque, myocardium or circulation and may be responsible for acute triggering of acute cardiovascular events e.g. strokes, arrhythmias and heart failure in these individuals. On the contrary, Pathway 1 (i.e. systemic inflammation) usually requires longer periods for activation of cellular inflammatory response (e.g. activated WBCs, platelets) and increased cytokines expression consequentially leading to atherosclerotic plaque vulnerability, enhanced coagulation & thrombotic changes, insulin resistance and dyslipidemia. These effects would predispose

individuals for future cardiovascular events, especially in those who already have traditional risk factors or may expedite acute effects via pathways 2 and 3 of later air pollutant exposures.

In general, there is a large degree of overlap among mechanisms and timing of physiological responses to PM inhalation. Also, response to PM inhalation may vary in relation to dosage, duration and chemical constituents of PM. However, there is an indication of strong overall mechanistic evidence in favor of pathway 1 (via systemic inflammation) as compared to other pathways in animal and human studies (Figure 1).

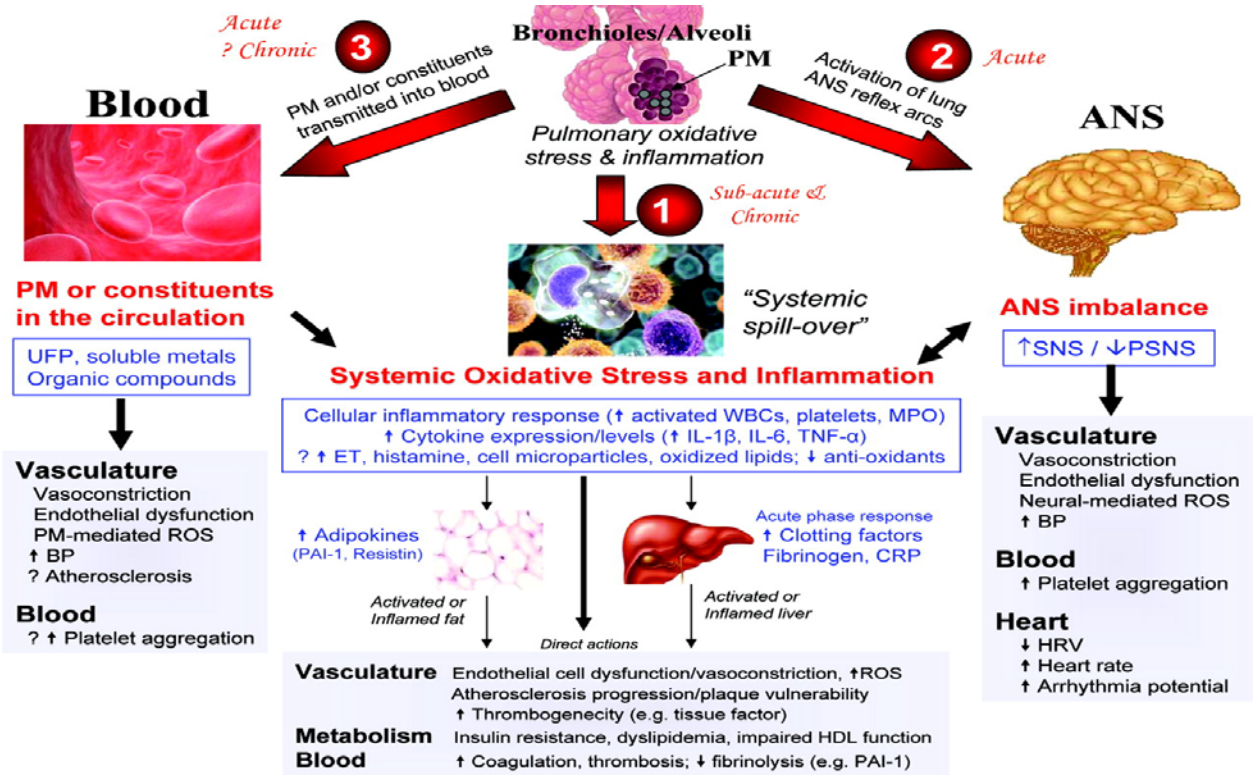

Figure 1: Biological pathways linking PM exposure with CVDs[a].

[a]Reprinted with permission Circulation.2010; 121:2331-2378 ©2010 American Heart Association, Inc.

1.6 SUMMARY

Over the last two decades, numerous studies in the United States and elsewhere have reported a positive relation between particulate air pollution and CVD morbidity and mortality. The epidemiologic evidence that explains biological mechanism behind this positive relationship comes from studies of blood markers of systemic inflammation (CRP and WBC), systemic oxidative stress (homocysteine) and thrombosis and coagulation (fibrinogen) and other blood markers in relation to particulate air pollution. However, due to errors in exposure assessment, different exposure time period, difference in PM constituents, relative small sample size and inadequate control of confounders, the findings of these studies are inconsistent. Thus, the results of my study that utilizes a large representative sample of US population with exposure assessment consistent with large populations at different lag periods may provide evidence of biological mechanism by which particulate air pollution is related to CVDs.

2.0 SPECIFIC AIMS

The specific aim and related hypothesis are:

Specific Aim 1: To examine the short term association of $PM_{2.5}$ air pollution exposure with cardiovascular mortality in Allegheny County, PA. We hypothesize that exposure to $PM_{2.5}$ air pollution exposure is positively associated with cardiovascular mortality.

Specific Aim 2: To examine the association of $PM_{2.5}$ air pollution exposure with biomarkers (i.e. CRP, WBC, Fibrinogen and HCY) of cardiovascular risk in adult NHANES participants. We hypothesize that exposure to $PM_{2.5}$ air pollution is positively associated with biomarkers of CVD risk

Specific Aim 3: To examine the association of $PM_{2.5}$ air pollution exposure with biomarkers (i.e. CRP, WBC, Fibrinogen and HCY) of cardiovascular risk in adult NHANES participants with and without metabolic syndrome. We hypothesize that participants with metabolic syndrome have increased response of change in biomarkers to $PM_{2.5}$ air pollution compared to participants without metabolic syndrome.

3.0 SHORT-TERM EXPOSURE TO $PM_{2.5}$ AIR POLLUTION AND CARDIOVASCULAR MORTALITY IN ALLEGHENY COUNTY, PA: USING A SPATIO-TEMPORAL KRIGING METHOD OF EXPOSURE ASSESSMENT

3.1 ABSTRACT

Background and Objectives: Short term exposure to fine particulate matter ($PM_{2.5}$) has been associated with cardiovascular diseases (CVD) mortality in numerous studies. However, findings from previous studies in Pittsburgh, Allegheny County, PA do not provide conclusive evidence of association of $PM_{2.5}$ with CVD mortality. Our objective was to estimate the relationship of short term exposure to $PM_{2.5}$ and CVD mortality in Allegheny County for the period 1999-2011. We also sought to identify vulnerable population subgroups based on sociodemographic characteristics, location at the time of death and time of year (season) of death.

Methods: We utilized a time-stratified case-crossover design to analyze natural CVD mortality and other specific natural CVD mortality outcomes i.e. ischemic heart disease (IHD), acute myocardial infarction (AMI), cerebrovascular disease, peripheral vascular disease (PVD), heart failure (HF) and cardiac arrhythmias for associations with $PM_{2.5}$, after adjusting for O_3 and apparent mean temperature. Stratified analysis was conducted for age, gender, race, education, season, and location at the time of death. Results are expressed as the percentage change in risk of mortality per 10 $\mu g/m^3$ increase for $PM_{2.5}$.

Results: We found a significant association of $PM_{2.5}$ with IHD {(2.1% (95% CI, 0.2 %-4.1%)} and PVD {(7.6% (95 % CI, 0%-15.7%)} mortality at lag day 5, adjusting for O_3 and apparent mean temperature. No significant association of $PM_{2.5}$ with AMI, cerebrovascular disease, HF or cardiac arrhythmia was observed. The risk of IHD mortality due to $PM_{2.5}$ was significantly different for those who died outside (4.9 %; 95 % CI, 1.3%-8.6%) of a hospital (0.8 %; 95 % CI, -2.1%-3.7%) or a nursing home (1.3 %; 95 % CI, -2.4%-5.0%). Those who died outside of hospital or nursing home mainly consisted of deaths at residence.

Conclusions: $PM_{2.5}$ air pollution was significantly associated with IHD and PVD mortality. The risk of IHD mortality due to $PM_{2.5}$ was significantly greater for individuals who died outside of a hospital or nursing home compared to deaths in the hospital or nursing home. This may reflect a more accurate real time exposure to event phenomenon based on spatiotemporal kriging method of exposure assessment at zip code of residence and should be explored further.

3.2 INTRODUCTION

Several studies have examined the relationship of short term ambient particulate matter ($PM_{2.5}$) air pollution with cardiovascular disease (CVD) mortality [9, 67-71]. Additionally, many studies have evaluated susceptibility (e.g. older people) to CVD mortality associated with short-term exposure to ($PM_{2.5}$) air pollution [70-72]. However, findings from previous studies in Pittsburgh, Allegheny County, do not provide conclusive evidence of association of $PM_{2.5}$ air pollution with CVD mortality [9-11]. These studies utilized exposure assessments based on single/limited monitors for the region.

Allegheny County has made considerable progress in reducing $PM_{2.5}$ air pollution in last decade, however, it is important to examine association of $PM_{2.5}$ air pollution and CVD mortality at current levels of air pollution and evaluate susceptible populations because estimates from other places may not be applicable to Allegheny County on account of differences in air pollution mixtures, population demographics and other factors. Further, none of the study conducted in Pittsburgh, Allegheny County examined the effects of $PM_{2.5}$ air pollution on CVD mortality by individual level characteristics such as age, gender, race, education, location at the time of death and time of year (season) of death.

The objectives of the present study were (1) to estimate the short term association between ambient particulate matter ($PM_{2.5}$) air pollution and cause specific mortality i.e., cardiovascular and other CVD outcomes, in Allegheny County, 1999-2011, while considering the latency of the association and confounding by ozone (O_3) and temperature; and (2) to identify vulnerable population subgroups based on sociodemographic characteristics, location at the time of death and time of year (season) of death. We utilized case-cross over design to analyze recent data (1999-2011) with a time frame of 13 years, to examine CVD mortality and other specific CVD mortality outcomes i.e. ischemic heart disease (IHD), acute myocardial infarction, cerebrovascular disease, peripheral vascular disease (PVD), heart failure and cardiac arrhythmias. Moreover, we employed spatiotemporal kriging at the ZIP Code Tabulation Areas (ZCTA) level to most precisely determine exposure levels of $PM_{2.5}$ and O_3.

3.3 MATERIALS AND METHODS

Air Pollution and Weather Data

ZCTA level estimates of $PM_{2.5}$and O_3 were obtained by spatiotemporal kriging with a product-sum covariance function using measurements from Air Quality System monitors in Allegheny County. The spatiotemporal kriging method has been widely used to interpolate spatiotemporal measurements of air pollutants. For a specific predicted point-value, the spatiotemporal kriging interpolation combined not only its spatially neighboring monitors but also measurements backward and forward within seven days. The overall accuracy of spatiotemporal Kriging was evaluated using 10-fold cross-validation. The amount of variance explained by $PM_{2.5}$and O_3 spatiotemporal kriging prediction models were 74.8 % and 87.9 % respectively. This was mainly limited due to less capture of less large-scale spatial variations [73].

Meteorological variables (mean air temperature, dew point temperature) were available from the Pittsburgh International airport. We calculated apparent air temperature as a combination of air temperature and dew point temperature to better take into account health effects of hot, humid days. The following formula was used to calculate apparent mean temperature:

*Apparent mean temperature (°c) = -2.653 + 0.994*Mean air temperature (°c) + 0.0153*(Dew point temperature (°c))* ^{2}

Mortality Data

Mortality data for Allegheny County residents were obtained from the Pennsylvania Department of Health Vital Statistics Division for January, 1999–December, 2011. Mortality data were collected from death certificate information and include, for each death, the date and cause of

death (International Classification of Diseases (ICD)-10), sex, age, race, education, location at the time of death, zip code of residence, county and state of death.

We classified natural cause mortality data into cardiovascular diseases of the circulatory system (ICD-10, I00-I99), ischemic heart disease (ICD-10, I20-I25), acute myocardial infarction (ICD-10, I21), cerebrovascular disease (ICD-10, I60-69), peripheral vascular disease (ICD-10, I70-79), heart failure (ICD-10, I50) and cardiac arrhythmias (ICD-10, I47-I49). Mortality data were categorized by age (<80, and ≥80 years, and missing), educational level (<12, 12, >12 years, and missing), race (white, black, other, and unknown), location at the time of death (hospital, nursing home and outside of hospital or nursing home), and season (cold (October-March), warm (April-September).

Statistical Analysis

A case-crossover design was utilized in which each case acts as his or her own control. This method offers the advantage of controlling for potential confounding from fixed or slowly varying individual-level characteristics. We used time-stratified referent selection, in which time is divided into fixed strata and the days in each stratum are considered for referents. Control days were matched on the same day of week in the same calendar month and year when a death (i.e., case) occurred. Cases had either 3 or 4 control days.

The $PM_{2.5}$ effects were examined with single-day (lag 0, 1, 2, 3, 4, 5) and unconstrained distributed day lags by cause-specific mortality. Lag 0 represents exposure on the same day as death; lag 1 represents exposure on the previous day, and so on, whereas unconstrained distributed day lag contains many lagged terms at the same time in model, so for example, the unconstrained distributed day lag model for maximum lag 5 will contain all lag terms from lag 0

to lag 5. All models were adjusted for ozone and apparent mean temperature at the same lag as $PM_{2.5}$ in single-day lag model or the same distributed lags in unconstrained distributed day lag models. We fitted conditional logistic regression models using PROC PHREG in SAS 9.3 (SAS Institute, Cary, NC, USA). Results are expressed as the percentage change in risk of mortality per 10 $\mu g/m^3$ increase for $PM_{2.5}$. To examine effect modification, we separately fitted models with an interaction term for $PM_{2.5}$and potential effect modifier (age, gender, race, education, season, and location at the time of death).

3.4 RESULTS

Around 60% of deaths were among individuals who were greater than 80 years of age for most of the outcomes except heart failure (75.2%) and cardiac arrhythmia (41.1%). A greater majority of events occurred among females ranging from 51.7%-62.5%. Approximately 90 % were of white race and roughly 10 % black for most of the outcomes, except for cardiac arrhythmia where blacks were in high proportions (17.5%). In terms of education level, the proportion of <12, 12 and >12 years of education were nearly quarter, half and quarter, except for the peripheral vascular diseases where around 50 % were >12 years educated. The majority (43% to 72%) of deaths occurred at a hospital. 53-55 % of deaths occurred in the cold season for all of the outcomes (Table 1).

The mean level of $PM_{2.5}$ ($\mu g/m^3$), O_3 (ppb), and apparent mean temperature (°C) were 13.8, 27.0 and 10.2 respectively for the entire study period. The pollutant levels were higher for the warm period compared to the cold period (Table 2). Shown in Table 3 are the distribution of the absolute differences between exposure on the case day and average of control days. The

correlation between pollutants and apparent mean temperature was low to moderate (Table 4). The time series of pollutants, apparent mean temperature and outcomes depict variation over the study period (Figure 2).

We estimated associations between daily concentrations of $PM_{2.5}$ at the zip code of residence and cause specific mortality for single and unconstrained distributed lag models. The single lag models were better as compared to distributed lag models because of small Akaike Information Criterion (AIC) of the single lag models. For subsequent analyses, we selected the single lag with the most certain effect estimate, as determined by the AIC, separately for each cause of death. We found significant association of $PM_{2.5}$ with IHD and PVD mortality at lag 5. For every 10 $\mu g/m^3$ increase in $PM_{2.5}$ at lag 5, there was a 2.1% (95% CI, 0.2 %-4.1%) increase in IHD mortality, whereas, for PVD, there was an increase of 7.6% (95 % CI, 0 %-15.7%), adjusting for O_3 and apparent mean temperature. There were no statistically significant associations of $PM_{2.5}$ with acute myocardial infraction, cerebrovascular disease, heart failure or cardiac arrhythmia (Figures 3 and 4).

The observed associations between $PM_{2.5}$ and cause-specific mortality were further investigated for effect modification by age, gender, race, education, location at the time of death and season. Table 5 shows the percent changes in mortality by cause, $PM_{2.5}$, and population subgroup associated with per 10 $\mu g/m^3$ increase in $PM_{2.5}$ for the selected lag. The risk of mortality was significantly different for those who died outside (4.9 %; 95 % CI, 1.3%-8.6%) of a hospital (0.8 %; 95 % CI, -2.1%-3.7%) or nursing home (1.3 %; 95 % CI, -2.4%-5.0%) for ischemic heart disease. Approximately 95% of deaths outside of a hospital or nursing home occurred at the residence. Except for this, we did not found any statistically significant interactions. However, we did found statistical significant effect estimates in different subgroups

for CVD, IHD and PVD mortality. For CVD mortality, the estimates were statistically significant for males (2.2%; 95 % CI, 0.1%, 4.4%), whites (1.7%; 95 % CI, 0.1%, 3.2%), outside of hospital or nursing home (3.8%; 95 % CI, 0.9%, 6.9%); and warm period (2.2%; 95 % CI, 0.2%, 4.2%). For IHD mortality, the estimates were statistically significant for >=80 years old (2.6%; 95 % CI, 0.1%, 5.2%), whites (2.3%; 95 % CI, 0.2%, 4.3%), outside of hospital or nursing home (4.9%; 95 % CI, 1.3%, 8.6%); and warm period (3.5%; 95 % CI, 0.7%, 6.5%). For PVD mortality, the estimates were statistically significant for male (13.7%; 95 % CI, 1.5%, 27.3%), hospital (11.6%; 95 % CI, 1.7%, 22.4%); and cold period (13.4 %; 95 % CI, 1.2%, 27.1 %) (Table 5).

3.5 DISCUSSION

Our study provides evidence of an association between short term exposure to ambient $PM_{2.5}$ (lag 5) and mortality due to IHD and PVD, after adjusting for O_3 and apparent mean temperature. The risk of dying due to IHD was significantly higher for those who died outside of a hospital or nursing home. For CVD mortality, the risk of dying was significant for males, whites, and in warm season whereas for IHD mortality, the risk of dying was significant for older, whites, and in warm season and for PVD mortality, the risk of dying was significant for males and in cold season.

The health impacts of particulate matter air pollution on mortality may differ for men and women primarily due to biological differences (hormonal complement, body size) or daily activity patterns. Literature shows weak evidence of higher particulate matter–associated risks for women than for men[74]. However, we found higher effect estimates for men compared to women for CVD and PVD mortality, although differences in effect estimates between sexes were

not statistically significant. Our study results are similar to a study conducted in Sao Paulo, Brazil [75].

There is strong evidence that older people are more susceptible to effects of air pollution [76]. Besides differences in physiology, older people likely have different indoor/outdoor activity patterns, occupational exposures, and social networks [76]. In our study, higher effect estimates for older people were observed for IHD mortality, though differences in effect estimates between older and younger people were not statistically different.

Evidence shows that exposure to air pollution and health status of populations, differs by race, with blacks more susceptible compared to other population groups [77, 78]. Differences in air pollution related mortality, similar to other health disparities, could be due to genetic differences among racial groups or race may acts as a surrogate marker for socioeconomic status among other reasons[79]. Most of the studies that evaluated role of race in impacting mortality due to particulate air pollution have found no statistical difference among racial groups [72, 80-82]. One of the limitation noted was use of simplistic categories of race i.e. % African Americans or dichotomous categories (e.g. Black, white) [76].We used racial categories as such i.e. white, black, other and unknown (% of other and unknown combined was <1%). In our study, higher effect estimates were observed among whites compared to black, others and unknown, although like previous studies these differences were not significant.

Socioeconomic status of an individual could modify particulate matter–associated health risks through differences in access to health care, baseline health status, occupational exposures, and nutrition. Education is the most commonly studied SES indicator for particulate matter–associated health risks. There is a suggestive evidence of higher risk of mortality with lower

education level [76]. We used education as an indicator for SES and did not found effect modification of education on mortality.

Seasonal differences in particulate matter–associated health risks have been observed in various studies [11, 83]. These differences may be due to differences in particulate matter constituents, level of exposure due to the rate of ventilation of indoor environments with outdoor concentrations, time spent outdoors and interaction between temperature and other weather conditions with particulate matter. In a seasonal analysis of particulate matter associated mortality, higher effect estimates were observed in spring and summer months in Northeast region of US [84].We found higher effects estimates for CVD and IHD mortality in summer season compared to winter season, though, differences in effect estimates were not statistically different. PM2.5 was associated with CVD mortality in the warm season at lag 0 and lag 1 day and in the cold season at lag 1 day in a study conducted in New York [4]. Another study found significant positive association for cumulative effect from lags 0 to 2 days for PM2.5 for all-cause and cardiovascular mortality in the warm season in Detroit; in contrast, Seattle showed positive associations in winter [5]. In the present study, risk of PVD mortality was higher and significant in winter period. This may be due to increased blood clotting because of cold and lower mobility in winter period.

Previous studies have found that risk of dying from air pollution is higher for deaths occurring out of hospital compared with in-hospital deaths [81, 85]. More than a threefold effect on all-cause mortality from high concentrations of PM_{10} was observed for deaths occurring out of hospital, when compared with in-hospital deaths, mainly driven by heart disease and stroke [81]. In the present study, risk of IHD mortality due to $PM_{2.5}$ was significantly greater for individuals who died outside of a hospital or nursing home compared to deaths in the hospital or nursing

home. This difference may be due to actual differences in exposure to air pollutants, as places other than hospital or nursing home may be less protected from ambient air pollution. Additionally, individuals at hospital or nursing home have obviously better access to treatment, thus alleviating effect of air pollution on mortality. We expect that people dying outside of a hospital or nursing home would be from disadvantaged groups e.g. blacks or homeless. These subpopulations may have difficulty in accessing timely health care leading to significantly higher effect of air pollution on CVD/ IHD mortality. The significantly higher estimates of those who died outside hospital or nursing home may be due to more accurate exposure assessment based on spatiotemporal kriging method at zip code of residence.

The present study had a sufficient sample size (except for cardiac arrhythmias) to examine multiple outcomes and effect modification by individual level characteristics. The large sample size was due to use of mortality data for whole Allegheny County for a time frame of 13 years. However, there are some limitations in our study. First, there may be exposure measurement error due to use of average zip code level exposure estimate for examining air pollution associated health risk, instead of individual level estimate. However, compared to studies that use a single central monitor for exposure estimate, average zip code level exposure estimates are better because they incorporates both spatial and temporal variations. Second, no association of PM2.5 air pollution with acute myocardial infarction, cerebrovascular disease, heart failure or cardiac arrhythmias were found. This might be due to low predictive power of spatiotemporal kriging prediction models used in this study. However, with the same model, we were able to detect association for CVD, IHD and PVD mortality.

Conclusion

In summary, the study showed that short term exposure to ambient PM2.5 air pollution was significantly associated with IHD and PVD mortality. The risk of IHD mortality due to PM2.5 air pollution was significantly greater for individuals who died outside of a hospital or nursing home compared to deaths in the hospital or nursing home. This may reflect a more accurate real time exposure to event phenomenon based on spatiotemporal kriging method of exposure assessment at zip code of residence and should be explored further.

3.6 TABLE AND FIGURES

Table 1. Demographics, place and time of cause specific mortality in Allegheny County, PA (1 January 1999- 31 December 2011)

	Cardiovascular Disease		Ischemic Heart Disease		Acute Myocardial Infraction*		Cerebrovascular disease		Heart Failure		Peripheral Vascular disease		Cardiac arrhythmia	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Total	62515	100	34945	100	11303	100	10116	100	4358	100	2256	100	992	100
Age (years)														
<80	25545	40.9	14920	42.7	4919	43.5	3622	35.8	1079	24.8	929	41.2	584	58.9
>=80	36966	59.1	20022	57.3	6382	56.5	6493	64.2	3279	75.2	1327	58.8	408	41.1
Missing	4	0.0	3	0.0	2	0.0	1	0.0	0	0	0	0	0	0
Gender														
Male	28096	44.9	16880	48.3	5132	45.4	3794	37.5	1699	39.0	942	41.8	384	38.7
Female	34419	55.1	18065	51.7	6171	54.6	6322	62.5	2659	61.0	1314	58.2	608	61.3
Race														
White	55739	89.2	31499	90.1	10241	90.6	8952	88.5	3967	91.0	2039	90.4	815	82.2
Black	6571	10.5	3332	9.5	1032	9.1	1126	11.1	377	8.7	210	9.3	174	17.5
Others	173	0.3	96	0.3	27	0.2	36	0.4	12	0.3	5	0.2	2	0.2
Unknown	329	0.1	18	0.1	3	0.0	2	0.0	2	0.1	2	0.1	1	0.1
Education (years)														
<12	15663	25.1	8790	25.2	2772	24.5	2407	23.8	1318	30.2	62	2.8	211	21.3
12	31760	50.8	17820	51.0	5921	52.4	5187	51.3	2064	47.4	612	27.1	508	51.2
>12	13597	21.8	7465	21.4	2368	21.0	2319	22.9	864	19.8	1135	50.3	257	25.9
Missing	1495	2.39	870	2.5	242	2.1	203	2.0	112	2.6	447	19.8	16	1.6
Location of death														
Hospital	29274	46.8	15105	43.2	6165	54.5	5318	52.6	1848	42.4	1383	61.3	723	72.9
Nursing home	18116	29.0	9522	27.3	2516	22.3	3826	37.8	1867	42.8	570	25.3	116	11.7
Outside of hospital or nursing home**	15125	24.2	10318	29.5	2622	23.2	972	9.6	643	14.8	303	13.4	153	15.4
Location of death														
Hospital, inpatient	22678	36.3	10442	29.9	4264	37.7	5018	49.6	1696	38.9	1126	49.9	618	62.3
Hospital/ER, outpatient	6449	10.3	4551	13.0	1849	16.4	295	2.9	148	3.4	252	11.2	104	10.5
Hospital, dead upon	139	0.2	106	0.3	48	0.4	5	0.1	4	0.1	4	0.2	1	0.1
arrival	8	0.0	6	0.0	4	0.0	0	0	0	0	1	0.0	0	0
Hospital, unknown														
Nursing Home	18116	29.0	9522	27.3	2516	22.3	3826	37.8	1867	42.8	570	25.3	116	11.7
Residence	14363	23.0	9813	28.1	2535	22.4	923	9.1	613	14.1	289	12.8	146	14.7
Other	762	1.2	505	1.5	87	0.8	49	0.5	30	0.7	14	0.6	7	0.7
Season														
Cold	33350	53.4	18659	53.4	6056	53.6	5439	53.8	2319	53.2	1218	54.0	551	55.5
Warm	29165	46.7	16286	46.6	5247	46.4	4677	46.2	2039	46.8	1038	46.0	441	44.5

*Mortality due to Acute Myocardial Infraction is a category of Ischemic Heart Disease

** Outside of hospital or nursing home** (includes mainly deaths at residence)

Table 2. Air pollutants levels and apparent mean temperature in Allegheny County, PA (1 January 1999- 31 December 2011)

	Entire Year					Warm Period					Cold Period				
	Mean	SD	Min	Max	IQR	Mean	SD	Min	Max	IQR	Mean	SD	Min	Max	IQR
$PM_{2.5}$ ($\mu g/m^3$)	13.8	7.6	0.8	64.5	8.9	15.7	8.6	1.2	64.5	10.7	12.0	5.8	0.8	49.8	7.4
O_3 (ppb)	27.0	9.8	0.1	83.3	14.4	32.8	8.1	3.5	83.3	10.8	21.3	7.6	0.1	65.9	10.3
Apparent Mean Temperature (°C)	10.2	11.0	-12.6	35.4	19.3	18.7	7.1	-4.3	35.4	10.1	1.7	7.0	-12.6	25.7	10.0

Table 3. Distribution of the absolute differences between exposure on case day and average of control days in Allegheny County, PA (1 January 1999- 31 December 2011)

Variable	Mean	Min	5^{th}	25^{th}	50^{th}	75^{th}	95^{th}	Max
$PM_{2.5}$ ($\mu g/m^3$)	6.0	0.0	0.4	2.2	4.7	8.4	15.7	43.5
O_3 (ppb)	5.8	0.0	0.4	2.2	4.8	8.2	14.9	36.7
Apparent Mean Temperature (°C)	4.6	0.0	0.3	1.9	3.9	6.6	11.6	22.8

Table 4. Spearman Correlation between pollutants and apparent mean temperature in Allegheny County, PA (1 January 1999- 31 December 2011), N=265064

	$PM_{2.5}$ ($\mu g/m^3$)	O_3 (ppb)	Apparent mean temperature (°C)
$PM_{2.5}$ ($\mu g/m^3$)	1.000	0.151 <.0001	0.371 <.0001
O_3 (ppb)	0.151 <.0001	1.000	0.532 <.0001
Apparent mean temperature (°C)	0.371 <.0001	0.532 <.0001	1.000

Table 5. Effect modification of Association between PM2.5 and mortality by cause of death, adjusting for O3 and apparent mean temperature by individual level characteristics and season, in Allegheny County, PA (1 January 1999-31 December 2011)

	Cardiovascular Disease (Lag5)			Ischemic Heart Disease (Lag5)			Acute Myocardial Infraction (Lag1)			Cerebrovascular disease (Lag2)			Heart Failure (Lag2)			Peripheral Vascular disease (Lag5)			Cardiac arrhythmia (Lag1)		
	%	95 % CI		%	95 % CI		%	95 % CI		%	95 % CI		%	95 % CI		%	95 % CI		%	95 % CI	
Age (years)																					
<80	1.4	-0.8	3.6	1.4	-1.5	4.4	0	-4.8	5.1	-0.8	-6.5	5.2	-2.7	-12.9	8.7	8.6	-2.8	21.4	-9.3	-22.2	5.7
>=80	1.0	-0.8	2.9	2.6	0.1	5.2	0.4	-4	4.9	1.7	-2.7	6.3	-3.6	-9.5	2.7	6.6	-3.3	17.4	-15	-29.3	2.2
Missing	84.9	-69.6	1023.3	576.6	-75.4	18512	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gender																					
Male	2.2	0.1	4.4	2.7	-0.1	5.5	2.4	-2.5	7.5	0.7	-4.9	6.7	-5.1	-13.1	3.6	13.7	1.5	27.3	-19.4	-33.5	-2.3
Female	0.3	-1.6	2.3	1.6	-1.1	4.3	-1.7	-6	2.8	0.8	-3.6	5.5	-2.2	-8.9	4.9	3.7	-5.8	14.1	-6.1	-19.1	9
Race																					
White	1.7	0.1	3.2	2.3	0.2	4.3	0.8	-2.6	4.4	1.2	-2.6	5.1	-3.4	-8.8	2.3	6.2	-1.7	14.6	-10	-20.7	2.3
Black	-2.	-6.8	1.7	1.1	-4.9	7.4	-6.3	-15.9	4.4	0.0	-10	11.0	-2.2	-18.5	17.3	26.9	-0.6	61.9	-23.6	-43.9	4.2
Others	-1	-24.6	29.9	-23	-49	16.2	10.6	-45.9	125.8	-48.7	-75.4	6.7	-97.1	-99.9	4.9	-32.5	-83.9	182.3	-	-	-
Unknown	-13.1	-55.9	71.2	27.1	-44.8	192.5	-73.1	-98.8	520.5	-	-	-	199.5	-97.5	35765.9	-46.4	-93.4	334	-	-	-
Education (years)																					
<12	1.5	-1.3	4.4	0.7	-3	4.6	-5.1	-11.3	1.4	-0.9	-7.7	6.5	-4.1	-13.1	6	-1.1	-14.2	14	-9.5	-29.3	15.8
12	0.7	-1.2	2.8	2.7	0.0	5.5	1.1	-3.4	5.8	0.8	-4.1	5.9	-5.6	-12.8	2.3	9.1	-1.5	20.8	-14.5	-27.6	1
>12	1.5	-1.6	4.7	1.9	-2.2	6.2	3.9	-3.4	11.7	1.9	-5.4	9.8	5.8	-6.5	19.6	14.1	-3.3	34.5	-8	-27.3	16.3
Missing	3.7	-5.3	13.4	5.0	-6.5	18	4.6	-16.6	31.3	8.7	-15.1	39.2	-15.6	-40.6	19.7	22.2	-20	86.5	2.9	-63.1	186.8
Location of death																					
Hospital	0.8	-1.3	2.9	0.8	-2.1	3.7	0.6	-3.7	5.2	-1.4	-6.1	3.5	-8.5	-16	-0.3	11.6	1.7	22.4	-11.2	-22.5	1.8
Nursing home	-0.3	-2.9	2.4	1.3	-2.4	5	-6	-12.5	1.1	5	-0.9	11.3	3.1	-5	11.8	3.2	-10.8	19.4	-4.6	-32.4	34.5
Outside of hospital or nursing home*	3.8	0.9	6.9	**4.9**	**1.3**	**8.6***	4.7	-2.1	12	-2.7	-13.1	8.9	-8.3	-20.8	6.2	-2.4	-20.5	19.8	-20.5	-41.9	8.7
Season																					
Cold	0.8	-1.4	3	0.8	-2.3	3.9	0	-5.2	5.5	-0.1	-5.7	5.7	-4.6	-12.7	4.4	13.4	1.2	27.1	-13.8	-28.5	3.9
Warm	2.2	0.2	4.2	3.5	0.7	6.5	-1	-5.7	3.9	1.2	-4.0	6.6	-3.6	-11.1	4.5	4.5	-6.3	16.5	-8.9	-23.4	8.3

*% risk was significantly higher for those who died outside of a hospital or nursing home for Ischemic Heart Disease mortality (P value for interaction <0.05).

- No estimate for these subgroups either due to no observations or models did not converge

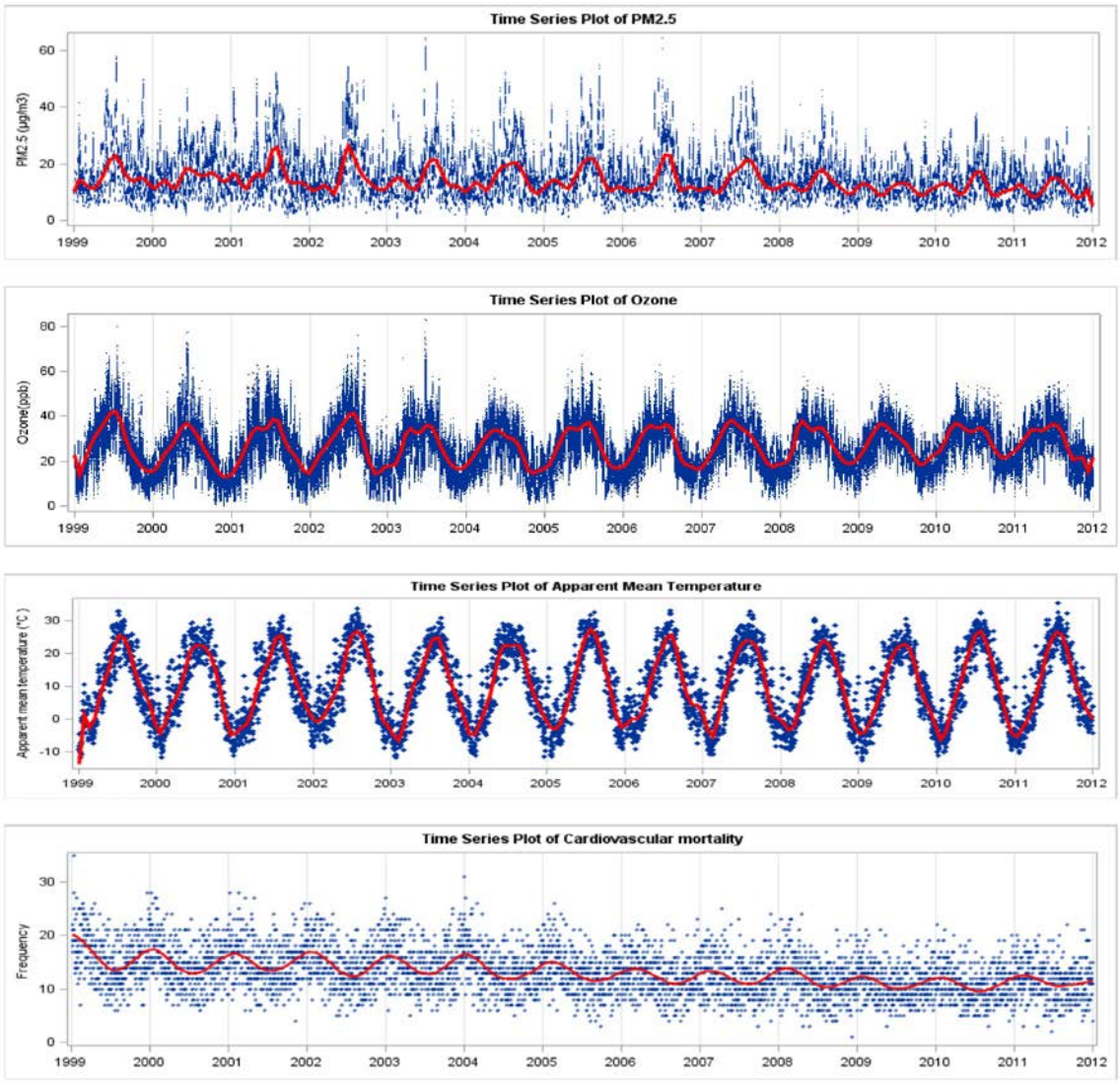

Figure 2. Time series of pollutants, apparent mean temperature and cardiovascular mortality in Allegheny County, PA (1 January 1999-31 December 2011)

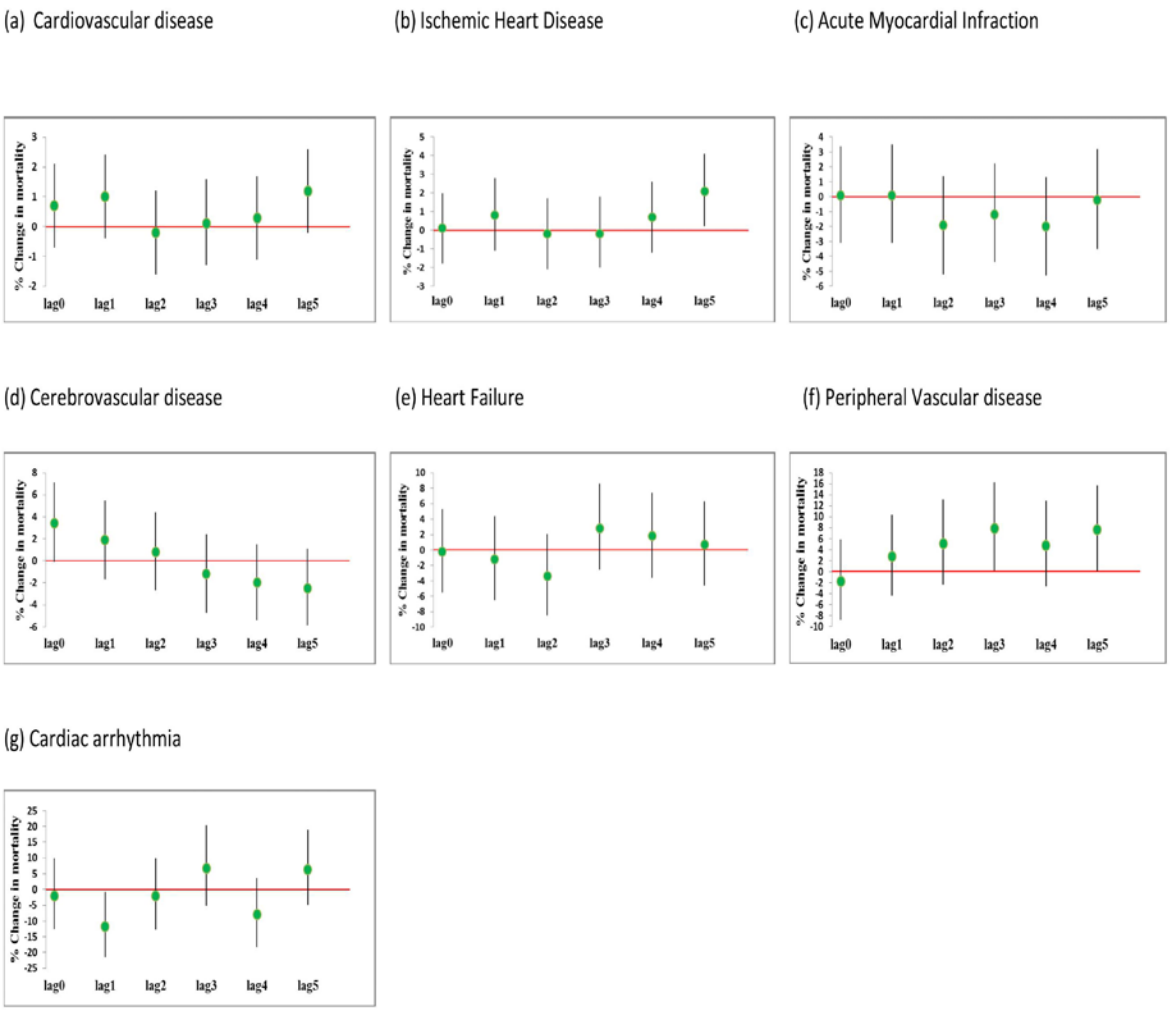

Figure 3. Association between PM2.5 and mortality by cause of death in Allegheny County, PA, expressed as percentage increase in risk (%) and 95% confidence intervals per 10 µg/m3 in single daily lag0 to lag5 (1 January 1999-31 December 2011), adjusted for O3 and apparent mean temperature

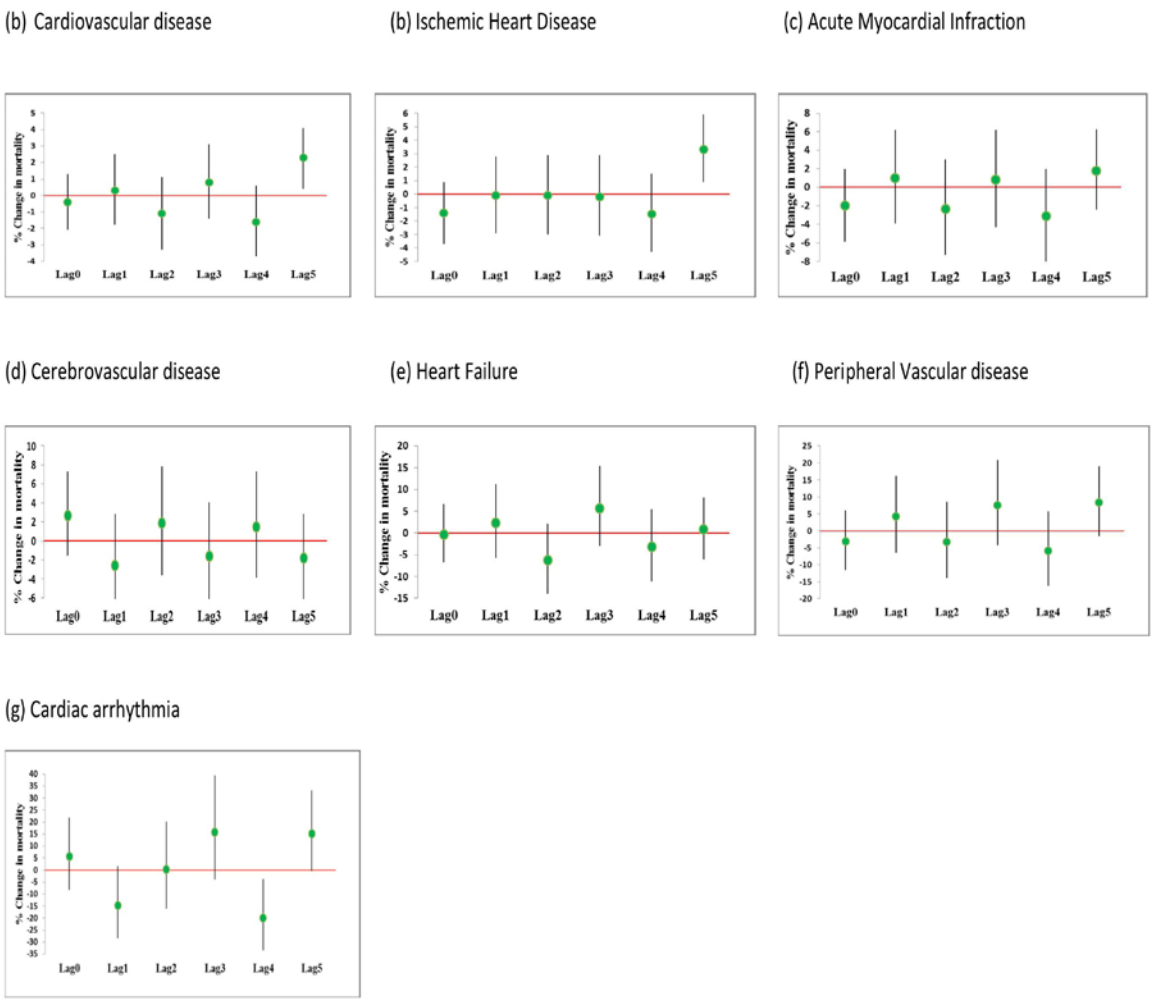

Figure 4. Association between PM2.5 and mortality by cause of death in Allegheny County, PA, expressed as percentage increase in risk (%) and 95% confidence intervals per 10 µg/m3 in unconstrained distributed lag models (1 January 1999-31 December 2011), adjusted for O_3 and apparent mean temperature

4.0 ASSOCIATION OF EXPOSURE TO PARTICULATE MATTER (PM2.5) AIR POLLUTION AND BIOMARKERS OF CARDIOVASCULAR DISEASE RISK IN ADULT NHANES PARTICIPANTS

4.1 ABSTRACT

Background and Objectives: Exposure to particulate matter ($PM_{2.5}$) is associated with increased cardiovascular mortality and morbidity, mediated by a hypothesized biological mechanism of systemic inflammation and oxidation. Our objective was to examine the association of ambient $PM_{2.5}$ exposure and markers of systemic inflammation and oxidation in adult National Health and Nutrition Examination Survey (NHANES) participants and within sensitive subgroups.

Methods: NHANES data (2001-08) on adult participants were merged with meteorological data from CDC WONDER and downscaler modelled air pollution data from the United States Environmental Protection Agency for each census tract in the 48 conterminous United States. The effects of short term (lags 0 to 3 days and their averages), and long term (30 & 60 day moving average and annual average (anavg)) $PM_{2.5}$ exposures on C-reactive protein (CRP, n=16160), white blood cells (n=16136), fibrinogen (n=2461) and homocysteine (n=11224) levels were analyzed using multiple linear regression, adjusting for age, gender, race, education, body-mass index (BMI), smoking status, total cholesterol, HDL cholesterol, diabetes, hypertension,

history of any cardiovascular disease, maximum apparent temperature and ozone. SAS SURVEYREG was used to account for the complex survey design of NHANES. Stratified analyses were conducted for BMI, diabetes, hypertension and smoking status.

Results: Overall, we found no statistically significant positive associations of either short term or long term $PM_{2.5}$ air pollution for any of the biomarkers after controlling for confounders. However, we found evidence suggesting stronger associations in participants with obesity, diabetes, hypertension and smokers. For every 10 $\mu g/m^3$ increase in $PM_{2.5}$, there was a significant increase of (a) 36.9 % (0.1 %, 87.2%) in CRP at anavg $PM_{2.5}$ (adjusted for short term exposure of $PM_{2.5}$ and O_3) among diabetics (b) 2.6 % (0.1 %, 5.1%) in homocysteine at lag 0 among smokers.

Conclusions: Although, we found no association between $PM_{2.5}$ and biomarkers of cardiovascular risk in general NHANES participants, there were subgroups that manifested increase in markers of systemic inflammation and oxidation to $PM_{2.5}$ exposure. Further studies should concentrate on the impact of $PM_{2.5}$ on markers of systemic inflammation and oxidation in those with multiple pre-existing cardiovascular risk factors.

4.2 INTRODUCTION

Numerous epidemiological studies have consistently shown that ambient particulate matter is associated with increased risk of cardiovascular mortality and morbidity [3, 54, 86]. Particles less than 10 micrometers in diameter (PM_{10}) have primarily been used as an exposure metric of particulate air pollution in previous studies [54] . However, fine particles, defined as less than 2.5

micrometers in diameter ($PM_{2.5}$), have a smaller size and can penetrate deeper into the lungs than large particles and may therefore be of greater health concern [87-89].

One of the biological pathways linking particulate matter air pollution exposure with cardiovascular mortality and morbidity could be systemic inflammation and oxidative stress [3]. C-reactive protein (CRP), white blood cell (WBC) count, homocysteine and fibrinogen are markers of systemic inflammation and oxidation associated with cardiovascular diseases [12]. Although many studies have examined the association of short term exposure, defined as less than 30 days duration, of $PM_{2.5}$ with CRP and fibrinogen [48, 90], there are few studies associating $PM_{2.5}$ with WBC count [19] and homocysteine [44, 63, 91]. Further, there are only a few studies examining long term exposure, defined as greater than equal to 30 days duration, of $PM_{2.5}$ with CRP [49, 50, 90] and fibrinogen [20, 90]. No study has examined the association of long term exposure of $PM_{2.5}$ with WBC count and homocysteine. Therefore, the relationship between $PM_{2.5}$ and biomarkers of cardiovascular risk remain inconclusive.

To date, two studies have been published that utilized nationwide National Health and Nutrition Examination Survey (NHANES) data in order to examine the effects of air pollution on biomarkers of cardiovascular risk, where PM_{10} was the exposure metric of particulate air pollution [54, 55]. Schwartz (2001) found positive associations for short term exposure of PM_{10} and fibrinogen and WBC counts. Chen et al. (2008) found significant positive association of long term PM_{10} and WBC count. However, to our knowledge, no study has been published examining the association of $PM_{2.5}$ and biomarkers of cardiovascular risk in a large nationally representative sample.

Our objective was to examine the association of ambient air pollution exposure with biomarkers of cardiovascular risk (CRP, WBC Count, homocysteine and fibrinogen) in adult

NHANES participants using nationally available modeled $PM_{2.5}$ data. We hypothesized that an increase in ambient $PM_{2.5}$ air pollution exposure would be associated with an increase in biomarkers of cardiovascular risk in adult NHANES participants, providing a potential explanation of the link between air pollution and cardiovascular disease.

4.3 MATERIALS AND METHODS

Health data. We used health data from the NHANES conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC) for the period 2001-2008. Details about this survey and the detailed measurement procedures and protocols have been described on the NCHS website [92]. In brief, the NHANES followed a complex, stratified, and multistage probability sampling of the population of the United States (US), with oversampling of minorities (African Americans and Mexican Americans) and the elderly (≥ 60 years of age). The survey consisted of an extensive household interview followed by a series of laboratory and other physical tests administered in a mobile examination center (MEC). Only those who completed the household interviews were invited for the MEC examination. Since 1999, NHANES has been continuously conducted in two year cycles. The survey was approved by the Institutional Review Board of the NCHS, and informed consent was obtained before participation. NHANES public use data sets were accessed for the four cycles of 2001-02, 2003-04, 2005-06, and 2007-08. We studied non-pregnant adults age 20 and older for CRP, WBC and homocysteine; and adults age 40 and older for fibrinogen. The data for CRP and WBC count were available from 2001 to 2008, homocysteine from 2001 to 2006 and fibrinogen from 2001-2002. After excluding pregnant women and participants with missing data on biomarkers levels

and covariates of interest, there were 16160, 16136, 11224 and 2461 participants included in our analysis for CRP, WBC, homocysteine and fibrinogen respectively.

Markers of Inflammation and covariates. The biomarkers of interest in this study were CRP, WBC count, homocysteine, and fibrinogen. In addition to these biomarkers, we also obtained data on demographic variables (including age, gender, race/ethnicity, and education) and potential risk factors for cardiovascular disease, namely smoking status, body mass index (BMI), total cholesterol, HDL cholesterol, self-report of physician diagnosed diabetes, history of any cardiovascular diseases (i.e. congestive heart failure, coronary heart diseases, angina/angina pectoris, heart attack, stroke) and hypertension. Smoking status was defined as follows: current- presently smoking cigarettes or serum cotinine levels were greater than or equal to 10 ng/mL; former- have smoked 100 cigarettes in life but currently not smoking; never- had not smoked at least 100 cigarettes in life. Hypertension was defined as systolic blood pressure $\geq$ 140 mmHg, diastolic blood pressure $\geq$ 90 mmHg, or a self-report of current use of antihypertensive medication. We also obtained data from NHANES on infections within the last 30 days (cold, gastrointestinal illness, flu/pneumonia/ear infection), chronic obstructive pulmonary disease (COPD) (includes emphysema and chronic bronchitis), household smoker presence and rheumatoid arthritis for conducting sensitivity analyses.

Ambient air pollution and weather data. Predictions of daily ambient 24-hour average $PM_{2.5}$ ($\mu g/m^3$) and 8-hour maximum O_3 levels (ppb) were obtained from the Environmental Protection Agency (EPA) using a downscaling modeling approach [93]. This downscaling approach uses Bayesian space-time modeling to combine air monitoring data and gridded numerical output from the Community Multi-Scale Air Quality Model (CMAQ) to produce point level daily air pollution predictions to the year 2000 US census tract centroids [94]. Daily

predictions of O_3 and $PM_{2.5}$ were obtained from January 1, 2001 – December 31, 2008 at the population weighted centroid (centers of population) of each year 2000 US census tract in the 48 conterminous states [95].

Meteorological data were obtained from the CDC WONDER North America Land Data Assimilation System Daily Air Temperatures and Heat Index (1979-2010) website [96]. Daily values of the maximum air temperature and maximum heat index for each county were extracted for the time period January 1, 2001 through December 31, 2008. Heat index incorporates both temperature and relative humidity and is a better measure on days when air temperature >80 F°. Maximum heat index was provided for those days when air temperature was above 80 F° or 26.7° C. CDC used a formula by Steadman to calculate the hourly heat index, from which the daily maximum heat index was computed [97]. For our analysis, we computed a daily maximum apparent air temperature which was defined as the daily maximum heat index if provided; otherwise the daily maximum air temperature was used.

We assembled an environmental database of daily pollution data and meteorological data for each census tract in the 48 conterminous United States for the time period January 1, 2001 through December 31, 2008. This large database contained predicted values of $PM_{2.5}$ and O_3 at the population weighted centroid of each year 2000 US census tract and maximum apparent temperature for each county assigned to the appropriate census tract level. In addition to the daily levels (lag 0), we calculated the following for $PM_{2.5}$ and O_3,: the level on the previous day (lag 1); two days prior (lag 2); three days prior (lag 3); the average of lags 0 and 1 (lag 0 to 1); average of lags 0, 1 and 2 (lag 0 to 2); average of lags 0, 1, 2, and 3 (lag 0 to 3); average of lags 1 and 2 (lag 1 to 2); and the average of lags 1, 2, and 3 (lag 1 to 3). The following long term

averages were also calculated: the average of the 30 days prior (30-day moving average), 60 days prior (60-day moving average), and annual average.

Merging of health and environmental data. The Census tract (11 digit Federal Information Processing Standards code) of residence of each individual and the date of the NHANES examination were used to merge the NHANES data with the environmental dataset of air pollution and weather described above. Thus, each NHANES participant was assigned $PM_{2.5,}$ $O_{3,}$ and temperature exposure based on the census tract of residence.

Statistical analysis. We performed weighted descriptive analyses (mean and standard error) for each biomarker overall and also stratified by covariates. We examined exposure to ambient $PM_{2.5}$ as a predictor of each biomarker of interest - CRP, WBC, homocysteine, and fibrinogen in separate regression models. CRP and homocysteine were log transformed to improve normality and stabilize the variance. The short term effects of $PM_{2.5}$ were examined. We specifically analyzed the effect of $PM_{2.5}$ on the day of the blood draw (lag 0) as well on the day before (lag 1), two days before (lag 2), and three days before (lag 3) and averages of these time periods (lag 0 to 1, lag 0 to 2, lag 0 to 3, lag 1 to 2, and lag 1 to 3). In addition, we examined the long term effects of $PM_{2.5}$ on each biomarker by using the average $PM_{2.5}$ in the 30 days prior, 60 days prior and annual average value. We also examined the annual average $PM_{2.5}$ after adjusting for short term effects of air pollution (lag 0 to 3 of $PM_{2.5}$ and O_3).

We used multiple linear regression models to assess the association of $PM_{2.5}$ with each biomarker and calculated regression estimates for a 10 $\mu g/m^3$ increase in $PM_{2.5.}$ We adjusted regression estimates for selected covariates based on prior biological and epidemiological knowledge of major determinants of cardiovascular health. Age, BMI, total cholesterol and HDL cholesterol were treated as continuous variables, whereas gender, smoking (current smokers vs

never & former smoker), diabetes, hypertension and history of any cardiovascular disease were treated as dichotomous variables in the models. Race/ethnicity was categorized as Non-Hispanic White, Non-Hispanic Black, Mexican American, and Other Race. Education was categorized as 1-11th grade, high school grade/GED or some college, and college graduate. Both single pollutant and two pollutant models (adjusted for the co-pollutant O_3 at the same lag or average as $PM_{2.5}$) were evaluated. The short term models were adjusted for the same day maximum apparent temperature, whereas the long term models were adjusted for the 30 day moving average maximum apparent temperature. Quartiles of temperature were used to account for non-linear relationship of temperature with biomarkers.

Based on prior studies, we used subgroup analyses to assess effect modification by smoking status (current smokers, never and former smoker), BMI (< 30 kg/m^2, ≥ 30 kg/m^2), self-report of physician diagnosed diabetes (Yes/No), and hypertension (Yes/ No) [19, 20, 30]. Regression estimates were adjusted for O_3 and maximum apparent temperature and other covariates.

Sensitivity analyses. Certain medical conditions (e.g. rheumatoid arthritis and COPD), acute infections and presence of household smoking have been related to elevated levels of inflammatory markers [98-101]. Therefore, we investigated the sensitivity of our results to alternate ways of modelling by excluding people with history of (a) rheumatoid arthritis; (b) COPD; (c) acute infection in last 30 days; (d) household smoking. We also examined our results after controlling for season and year because temperature and pollutants show seasonal and yearly trend.

All statistical analyses were performed using SAS software, version 9.2, Cary, NC, US. Descriptive analyses were conducted using PROC SURVEYMEANS and PROC

UNIVARIATE. All regression models were run accounting for the complex sampling design of the NHANES with the SAS SURVEYREG command by using the sample weights included in the datasets. P-values <0.05 of were considered significant. All p-values were 2-tailed.

4.4 RESULTS

Table 6 shows the distribution of environmental variables from 2001 to 2008 at the level of participant's address on the day of blood draw. The mean ±standard error of $PM_{2.5}$ ($\mu g/m^3$), O_3 (ppb), and maximum apparent temperature (°C) were 11.88±0.37, 42.38 ±0.93, and 22.22±0.46 respectively.

Table 7 shows the survey weighted descriptive statistics of biomarkers for non-pregnant adult participants, excluding those with missing data on biomarkers levels and covariates of interest. Females had higher levels of CRP and fibrinogen and lower levels of homocysteine, whereas there were no gender differences in levels of WBC. Blacks had elevated levels of all the biomarkers except WBC. Older and less educated participants had raised levels of biomarkers. Biomarker levels were elevated in those with hypercholesterolemia, among current smokers, and those with the presence of diabetes, obesity, hypertension, history of any CVD, rheumatoid arthritis, COPD and recent infections (except homocysteine that was higher in former smokers, overweight and those without any infections in last 30 days).

In the complete study group, none of the $PM_{2.5}$ lags (short term or long term) were significantly positively associated for any of the biomarkers in both single pollutant and bi-pollutant models after adjusting for age, gender, race, education, BMI, smoking status, total

cholesterol, HDL cholesterol, diabetes, hypertension, history of any cardiovascular disease, maximum apparent temperature and ozone (Figure 5 and 6).

Effect modification by obesity, diabetes, hypertension and smoking

In subgroup analyses, we found increased response of change in biomarkers in participants who had obesity, diabetes, hypertension, and who were smokers, compared to those who did not have these cardiovascular risk factors (Figure 7, 8, 9 and 10). In particular, participants with diabetes showed consistent increased response across all biomarkers compared to participants without diabetes.

We found some significant and near significant positive associations for diabetic participants: (a) for every 10 $\mu g/m^3$ increase in annual average $PM_{2.5}$ (adjusted for short term exposure of $PM_{2.5}$ and O_3), there was a significant increase of 36.9 % (0.1 %-87.2%) in CRP; (b) for every 10 $\mu g/m^3$ increase in 60 day moving average $PM_{2.5,}$ there was a near significant increase of 0.3 x 10^3 cells /µL (-0.05 x 10^3 - 0.69 x 10^3) in WBC count (c) for every 10 $\mu g/m^3$ increase at 2 day lag $PM_{2.5,}$ there was a near significant increase of 5 % (-0.2%-10.5%) in homocysteine. Also, for smokers, there was a significant increase of 2.6 % (0.1 %-5.1%) in homocysteine for every 10 $\mu g/m^3$ increase at zero day lag $PM_{2.5}$.

Sensitivity Analysis

The results of sensitivity analyses excluding people with history of (a) rheumatoid arthritis; (b) COPD; (c) acute infection in last 30 days; (d) household smoking were largely similar. Also, adjusting for season and year led to similar results (Data not shown).

4.5 DISCUSSION

Our objective was to examine the association of $PM_{2.5}$ air pollution exposure with biomarkers of cardiovascular risk (specifically CRP, WBC, fibrinogen, and homocysteine) in adult NHANES participants. Overall, we found no statistically significant positive association of either short term or long term $PM_{2.5}$ air pollution with any of the biomarkers of cardiovascular risk. However, participants with obesity, diabetes, hypertension and smoking history showed increased levels of cardiovascular biomarkers with increase in $PM_{2.5}$ exposure, compared to those participants without these cardiovascular risk factors. In particular, participants with diabetes showed consistent increased response across all biomarkers compared to participants without diabetes. Our findings were robust to alternate ways of modelling conducted by excluding people with history of (a) rheumatoid arthritis; (b) COPD; (c) acute infection in last 30 days; (d) household smoking and to adjustment for season and year.

Short term effects of $PM_{2.5}$ air pollution on biomarkers

Our study supports largely null findings of previously conducted research among the general [44, 45, 48, 50, 90], elderly [48], and COPD [52] populations for short term effects of $PM_{2.5}$ on CRP. In contrast to our study, Meier et al. (2014) reported significant positive association of 15 hour averages of $PM_{2.5}$ on CRP in a panel study of non-smoking male highway maintenance workers [47]. Similarly, Zhao et al. (2013), in a cross-section of 101 25-55 years old non-smoking traffic policemen with no history of cardiopulmonary disease and no current medication in Shanghai, China, showed significant positive association of 24 hour averages of $PM_{2.5}$ on CRP [51]. In both studies, significant positive findings may be due to participants' regular exposure to very high level of air pollution on account of their occupation.

In regard to the association of $PM_{2.5}$ with fibrinogen, in concordance with our study, previous investigations of both general [27, 28, 44, 56, 58, 90, 102], elderly [19, 29, 48], and pre-existing COPD [52] and heart disease [35, 37, 57] populations have reported null findings.However, Ruckerl et al. (2007) reported significant positive association of lag 3 $PM_{2.5}$ in a prospective cohort of 1,003 Myocardial Infraction survivors in six European cities. This positive finding may be explained by exposure to high levels of air pollution in Myocardial Infraction survivors who are susceptible due to previous physiologic insult [38].

In regard to the association of $PM_{2.5}$ with WBC, our study is in agreement with null findings of previous studies among young [102], elderly [19, 25, 30], and elderly populations with preexisting heart disease [37]. In contrast to our study, Dubowsky et al. (2006) reported positive associations of $PM_{2.5}$ with WBC; these associations increased with longer moving average and reached statistical significance for previous moving average of 7 days in a panel study of 44 elderly participants. However, similar to our study they reported non-significant findings for moving averages larger than 14 days.

Out of three studies conducted so far examining the association of $PM_{2.5}$ with homocysteine, only Ren et al. (2010) reported significant positive associations of moving averages of 5, 6 and 7 days of $PM_{2.5}$ and homocysteine in the Normative Aging Study cohort of 1000 elderly non-Hispanic white community residing men [44, 63, 91]. Our study supports otherwise null findings.

Long term effects of $PM_{2.5}$ air pollution on biomarkers

The null association of a long term effect of $PM_{2.5}$ on CRP in our study is in accord with previous studies [48, 90] . In contrast to our study, Hennig et al. (2014) and Ostro et al. (2014) reported

significant positive association for prior year ambient $PM_{2.5}$ with CRP. However, similar to our study, Hennig et al. (2014) found no association for 28-day mean $PM_{2.5}$ .With regard to the association between long term effects of $PM_{2.5}$ on fibrinogen, our findings are consistent with three previous US studies [19, 48, 90]. However, Hoffmann et al. (2009) reported significant association for men in a cross-sectional study of 4032 participants from the densely populated and highly industrialized Ruhr area, Germany [20]. To our knowledge, ours is the first study to examine the association of long term $PM_{2.5}$ exposure on homocysteine and WBC, and we found no significant positive association.

Effect modification by obesity, diabetes, hypertension and smoking

We found suggestive evidence that participants with obesity, diabetes, hypertension and smokers experienced larger increase in biomarkers compared to participants without these cardiovascular risk factors. In particular, participants with diabetes showed consistently increased response across all biomarkers compared to participants without diabetes. This finding is in accord with previous studies of short term [30, 59] and long term air pollution [49] and our current understanding of susceptible population groups to air pollution [3].

Using NHANES and $PM_{10,}$ Schwartz (2001) and Chen et al. (2008) found positive associations for short term (same day) exposure with fibrinogen and WBC counts; long term (1 year) exposure and WBC count respectively. However, both studies utilized NHANES III (1989-1994) participants who lived in urban areas only and the pollutant data from US EPA Air Quality System monitors [54, 55]. PM_{10} levels during this earlier time period (1989-1994) were significantly higher (mean PM_{10} = 35.2 $\mu g/m^3$)[54].

Our findings are in accordance with Balmes et al. (2013), who utilized mixed model analysis to evaluate NHANES (2001-2006) participants from both rural and urban area and US EPA's hierarchical Bayesian modeled $PM_{2.5}$.They reported no significant positive associations for both short and long term $PM_{2.5}$ with CRP and WBC count. However, similar to our findings, they also reported larger increase in CRP and WBC count in participants with hypertension compared to those without hypertension (Balmes J, Mann J, Navarro K, McKone T, unpublished data).

Strengths and limitations

To our knowledge, this is the first nationwide population-based study examining the association of short and long-term exposure to $PM_{2.5}$ air pollution with biomarkers of cardiovascular risk. The $PM_{2.5}$ exposure was assessed by using pollutant predictions at the population weighted centroid of the census tract using downscaling model approach from EPA [93]. This approach allows use of health data for nearly the entire country instead of being limited to urban areas due to its ability to predict air pollutant concentrations for a large spatial extent and makes study findings generalizable to the US. Additionally, it better predicts temporal variability indicative of air pollutant concentrations measured at air quality monitors compared with earlier CMAQ models and spatial interpolation methods [93, 103]. Our study was able to consider health effects at the lower end of ambient particulate matter exposure compared to previous studies [54] . Our study suggests that even at low level of air pollution, those with multiple pre-existing cardiovascular risk factors might have an increased risk of cardiovascular disease when exposed to particulate matter air pollution.

This study must be interpreted in the context of its known limitations. There is a potential of exposure misclassification due to less confidence in the pollutant predictions in rural areas because of the increasing distance of these locations from air quality monitors [103]. Additionally, there is a possibility of error in exposure measurement due to use of average population exposure rather than individual exposure estimates and not accounting for the time spent indoors vs outdoors by the participants.

Conclusions

Overall, there were no appreciable effects of short and long-term exposure to $PM_{2.5}$ air pollution with regard to biomarkers of cardiovascular risk after adjusting for demographic and cardiovascular risk factors in our nationally representative sample of adult men and women. However, we did find some evidence suggesting stronger associations of $PM_{2.5}$ with biomarkers of cardiovascular risk in participants with elements of metabolic syndrome e.g. obesity, diabetes, hypertension and smokers. Further studies should concentrate on the impact of $PM_{2.5}$ on markers of systemic inflammation and oxidation in those with multiple pre-existing cardiovascular risk factors.

Acknowledgements

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4.6 TABLES AND FIGURES

Table 6. Distribution of environmental variables

Year	N	$PM_{2.5}(\mu g/m^3)$	O3 (ppb)	Temperature (° C)
2001	2227	11.02 ± 0.59	41.25 ± 1.53	23.18±1.33
2002	2130	12.64 ± 1.51	46.06 ± 3.18	23.12±1.19
2003	2018	12.77 ± 1.08	43.79 ± 2.50	22.18±1.00
2004	2175	11.32 ± 0.81	38.96 ± 1.82	22.37±1.58
2005	1893	12.69 ± 0.96	43.35 ± 2.84	21.36±1.40
2006	2074	10.83 ± 0.68	39.30 ± 1.69	21.23±1.01
2007	2604	12.80 ± 1.43	44.39 ± 2.84	22.46±1.83
2008	2667	11.37 ± 0.62	42.83 ± 2.17	21.93±1.22
2001-2008	17788	11.88 ± 0.37	42.38 ± 0.93	22.22±0.46

*Values are mean ± SE

Table 7. Survey weighted descriptive statistics of biomarkers for non-pregnant adult study participants

	C-reactive protein		White blood cells count		Homocysteine		Fibrinogen	
	n (%)	Mean±SE (mg/dL)	n (%)	Mean ± SE (10^3 cells/μL)	n (%)	Mean ± SE (μmol/L)	n (%)	Mean ± SE (mg/dL)
Overall	16160 (100)	0.40±0.01	16136(100)	7.26±0.03	11224(100)	8.80±0.07	2461(100)	368.54±4.74
Age (years)								
20-39	5227 (37.4)	0.35±0.01	5218(37.4)	7.40±0.04	3686(37.8)	7.72±0.06	N/A	N/A
40-59	5228 (39.9)	0.41±0.01	5223(39.9)	7.21±0.05	3611(39.9)	8.79±0.07	1252(67.0)	356.20±5.19
60+	5705 (22.8)	0.47±0.01	5695(22.8)	7.12±0.04	3927(22.3)	10.66±0.14	1209(33.0)	393.57±4.69
Gender								
Male	8234(49.7)	0.34±0.01	8219(49.6)	7.25±0.04	5782(50.0)	9.45±0.08	1255(49.4)	357.34±5.55
Female	7926(50.4)	0.46±0.01	7917(50.4)	7.27±0.04	5442(50.0)	8.16±0.09	1206(50.6)	379.47±4.62
Race/Ethnicity								
White	8340(72.6)	0.39±0.01	8326(72.6)	7.32±0.04	5941(73.2)	8.96±0.08	1433(78.6)	365.01±5.49
Black	3205(10.5)	0.51±0.02	3202(10.5)	6.59±0.05	2255(10.5)	8.94±0.11	429(8.7)	392.62±4.87
Hispanic	3097(7.6)	0.44±0.02	3096(7.6)	7.48±0.05	2246(7.4)	7.58±0.06	439(4.2)	367.91±2.57
Others	1518(9.3)	0.37±0.02	1512(9.3)	7.37±0.07	782(8.9)	8.39±0.15	160(8.5)	376.85±5.19
Education								
1-11th grade	4633(18.1)	0.47±0.02	4622(18.0)	7.54±0.06	3128(17.3)	9.21±0.15	710(17.3)	392.64±5.41
HS grad/GED	8329(56.0)	0.41±0.01	8319(56.0)	7.35±0.04	5840(56.6)	8.83±0.08	1200(53.4)	370.69±5.27
College graduate	3198(26.0)	0.32±0.01	3195(26.0)	6.88±0.04	2256(26.1)	8.47±0.09	551(29.3)	350.39±4.59
Smoking								
Never	8233(51.0)	0.38±0.01	8218(51.0)	6.92±0.03	5654(50.3)	8.39±0.06	1155(48.1)	363.76±4.05
Former	4232(24.8)	0.40±0.01	4226(24.7)	7.02±0.05	2984(24.8)	9.28±0.12	835(31.8)	367.16±6.05
Current	3695(24.4)	0.44±0.01	3692(24.4)	8.21±0.06	2586(25.0)	9.17±0.13	471(20.1)	382.17±7.35
BMI								
<25.0	4943(33.1)	0.26±0.01	4938(33.1)	6.96±0.04	3532(33.6)	8.49±0.09	693(29.3)	348.88±5.48
25-29.9	5779(34.9)	0.35±0.01	5767(34.9)	7.16±0.04	4063(34.9)	9.05±0.08	987(38.9)	364.05±4.21
>=30.0	5438(32.1	0.60±0.0	5431(32.1)	7.69±0.05	3629(31.5)	8.86±0.10	781(31.7)	392.23±4.75
Hypertension								
Yes	6006(30.2)	0.49±0.01	5996(30.2)	7.40±0.04	4101(29.8)	10.10±0.10	1177(39.8)	386.76±5.23
No	10154(69.8)	0.36±0.01	10140(69.8)	7.20±0.04	7123(70.2)	8.26±0.07	1284(60.2)	356.52±4.97
Total Cholesterol (mg/dL)								
<240	13627(84.2)	0.40±0.01	13606(84.2)	7.23±0.04	9436(83.8)	8.73±0.07	1971(79.9)	365.72±5.13
>=240	2533(15.8)	0.41±0.01	2530(15.8)	7.45±0.05	1788(16.2)	9.17±0.12	490(20.1)	379.77±4.32
HDL-Cholesterol (mg/dL)								
<40	3092(19.1)	0.49±0.02	3089(19.2)	7.87±0.06	2011(17.9)	9.05±0.10	500(19.9)	374.45±8.36
40-49	4671(28.4)	0.41±0.01	4662(28.4)	7.46±0.05	3289(28.7)	8.98±0.10	726(28.8)	376.00±5.26
50-59	3775(23.3)	0.39±0.02	3773(23.3)	7.08±0.05	2637(23.4)	8.65±0.08	539(22.0)	368.10±4.30
>60	4622(29.2)	0.34±0.01	4612(29.2)	6.82±0.04	3287(29.9)	8.61±0.10	696(29.3)	357.56±5.16
Diabetes								
Yes	1763(7.5)	0.57±0.03	1759(7.5)	7.62±0.08	1139(7.1)	10.10±0.20	318(9.5)	399.92±8.63
No	14397(92.5)	0.39±0.01	14377(92.5)	7.23±0.03	10085(92.9)	8.71±0.07	2143(90.5)	365.27±4.61
History of any CVD								
Yes	1867(8.4)	0.57±0.02	1864(8.4)	7.46±0.06	1299(8.4)	11.38±0.26	349(11.0)	403.86±6.76
No	14293(91.6)	0.39±0.01	14272(91.6)	7.24±0.03	9925(91.6)	8.57±0.06	2112(89.0)	364.17±5.04
Recent Infection								
Yes	4104(26.0)	0.53±0.02	4100(26.0)	7.51±0.05	2924(27.1)	8.70±0.12	615(24.3)	375.15±4.48
No	11273(74.0)	0.36±0.01	11256(74.0)	7.16±0.04	7736(73.0)	8.87±0.06	1785(75.7)	366.30±5.33
Rheumatoid Arthritis								
Yes	882(4.1)	0.60±0.03	880(4.1)	7.45±0.13	591(4.1)	9.73±0.28	161(5.0)	402.54±6.75
No	15252(95.9)	0.39±0.01	15230(95.9)	7.25±0.03	10613(95.9)	8.77±0.06	2296(95.01)	366.69±4.80
COPD								
Yes	1233(7.4)	0.61±0.03	1234(7.5)	7.77±0.10	816(7.4)	9.43±0.23	189(7.5)	401.13±7.54
No	14880(92.6)	0.38±0.01	14855(92.6)	7.22±0.03	10372(92.7)	8.75±0.07	2259(92.5)	365.60±4.88
Household Smoker								
Yes	3159(20.0)	0.48±0.02	3157(20.0)	8.07±0.07	2225(20.6)	9.47±0.14	466(19.5)	380.99±7.06
No	12883(80.0)	0.38±0.01	12861(80.0)	7.06±0.03	8906(79.4)	8.64±0.06	1971(80.5)	365.49±4.60

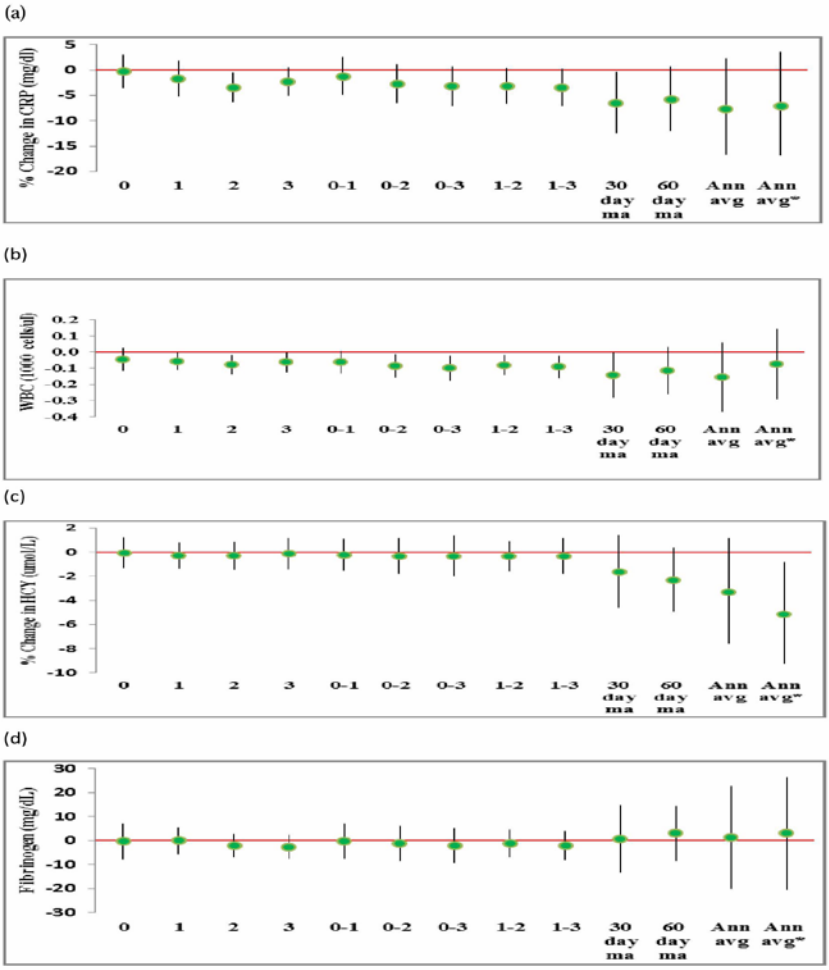

Figure 5. Change (95 % CI) in biomarkers for per 10 µg/m3 increase in PM2.5 at different lag times (a) C - reactive protein (CRP) (b) White Blood Cell (WBC) counts (c) Homocysteine (d) Fibrinogen. Models are adjusted for age, gender, race/ethnicity, education, smoking status, body mass index, total cholesterol, HDL cholesterol, diabetes, hypertension, history of any cardiovascular diseases and maximum apparent temperature.

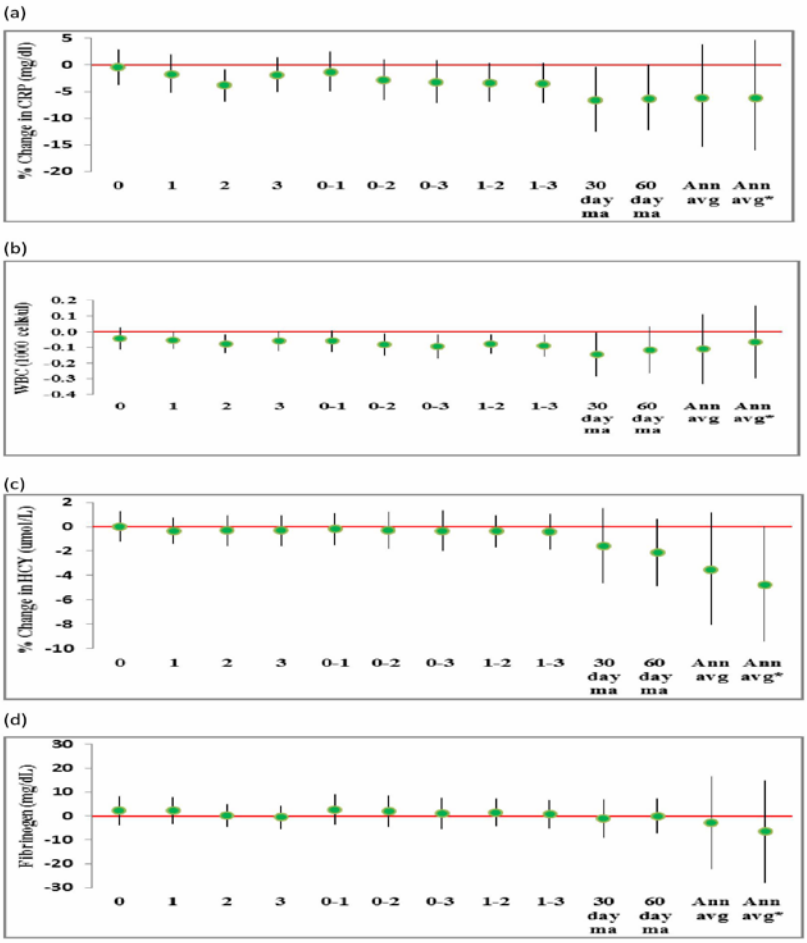

Figure 6. Change (95 % CI) in biomarkers for per 10 µg/m3 increase in PM2.5 at different lag times (a) C - reactive protein (CRP) (b) White Blood Cell (WBC) counts (c) Homocysteine (d) Fibrinogen. Models are adjusted for age, gender, race/ethnicity, education, smoking status, body mass index, total cholesterol, HDL cholesterol, diabetes, hypertension, history of any cardiovascular diseases, maximum apparent temperature and ozone

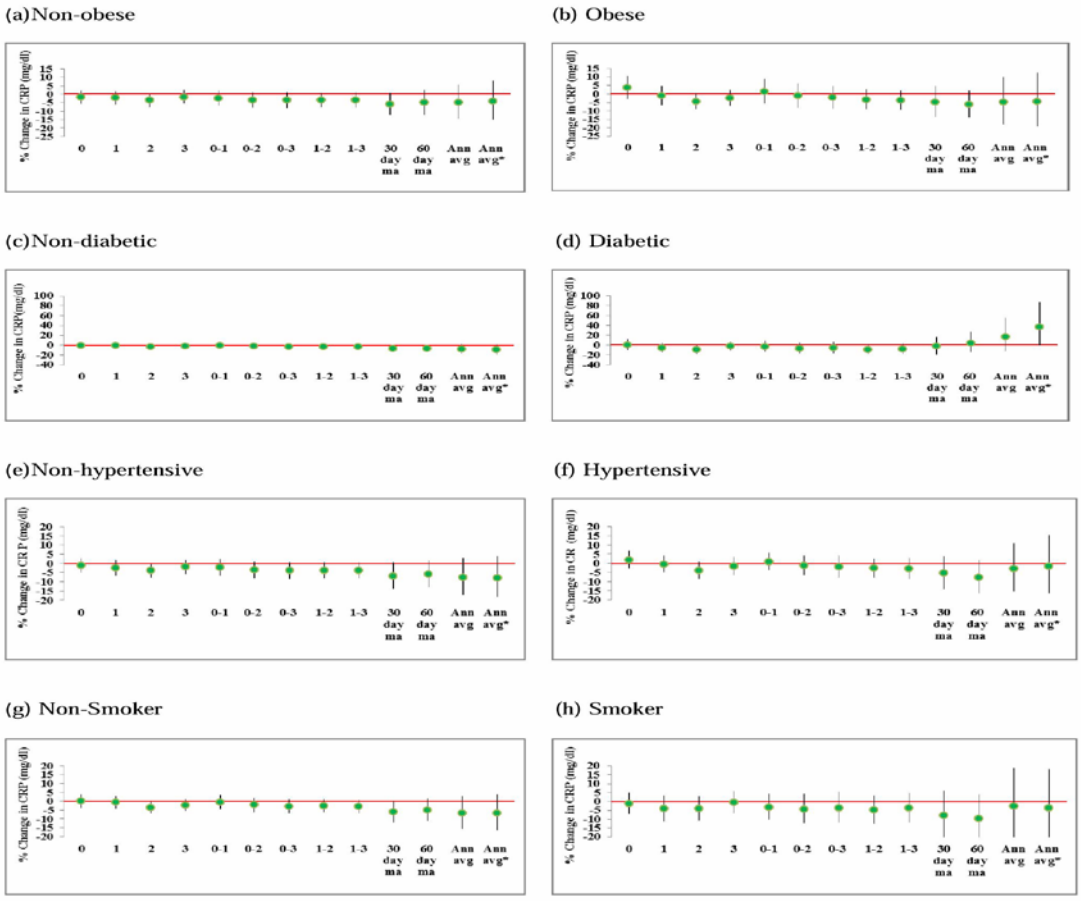

Figure 7. Effect Modification of associations between PM2.5 and C - reactive protein (CRP) by disease condition at different lag times. Models are adjusted for age, gender, race/ethnicity, education, smoking status, body mass index, total cholesterol, HDL cholesterol, diabetes, hypertension, history of any cardiovascular diseases and maximum apparent temperature and ozone.

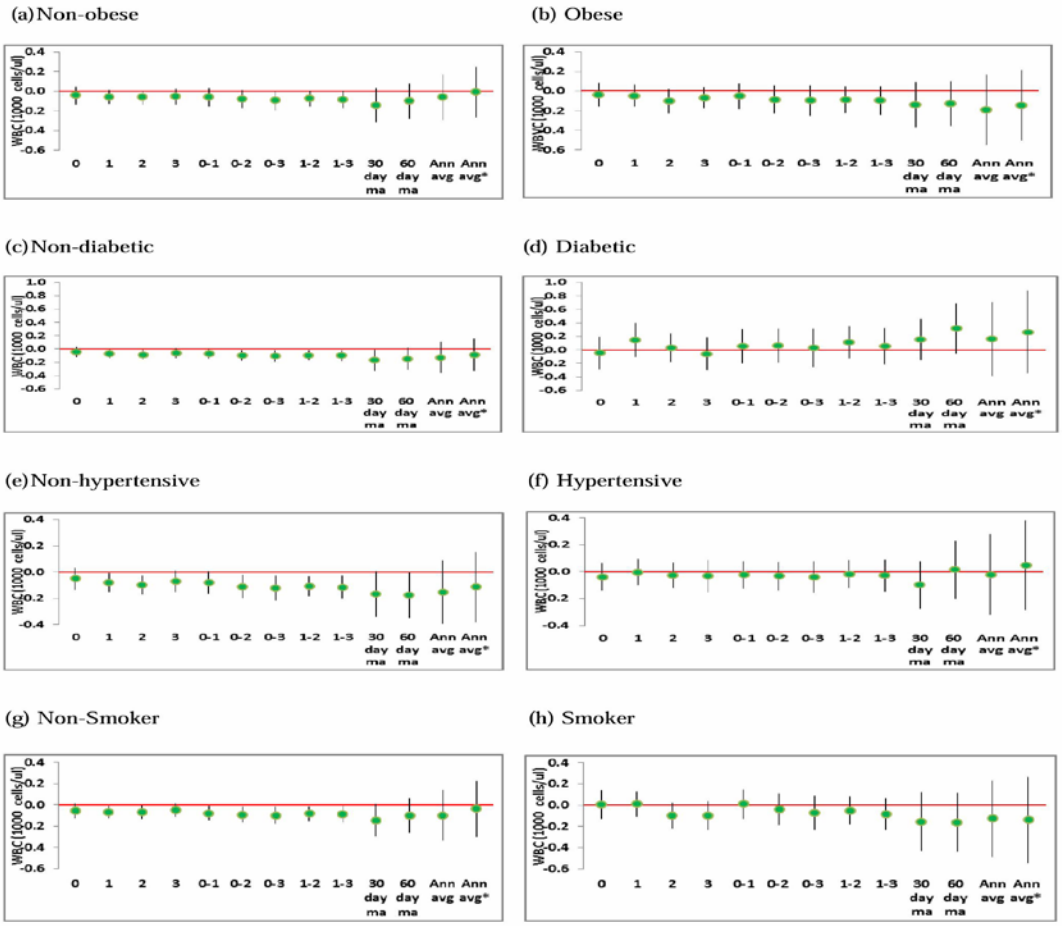

Figure 8. Effect Modification of associations between PM2.5 and White Blood Cell (WBC) counts by disease condition at different lag times. Models are adjusted for age, gender, race/ethnicity, education, smoking status, body mass index, total cholesterol, HDL cholesterol, diabetes, hypertension, history of any cardiovascular diseases and maximum apparent temperature and ozone.

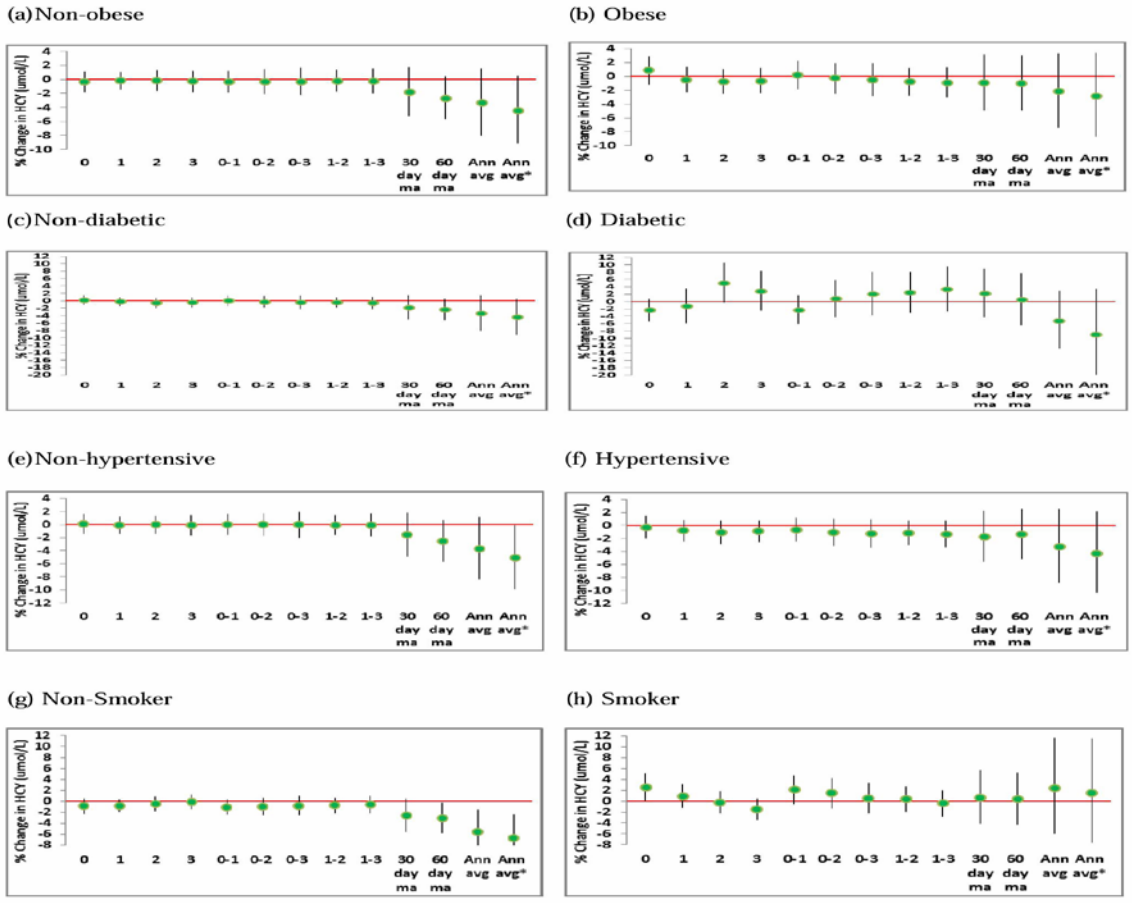

Figure 9. Effect Modification of associations between PM2.5 and Homocysteine by disease condition at different lag times. Models are adjusted for age, gender, race/ethnicity, education, smoking status, body mass index, total cholesterol, HDL cholesterol, diabetes, hypertension, history of any cardiovascular diseases, maximum apparent temperature and ozone.

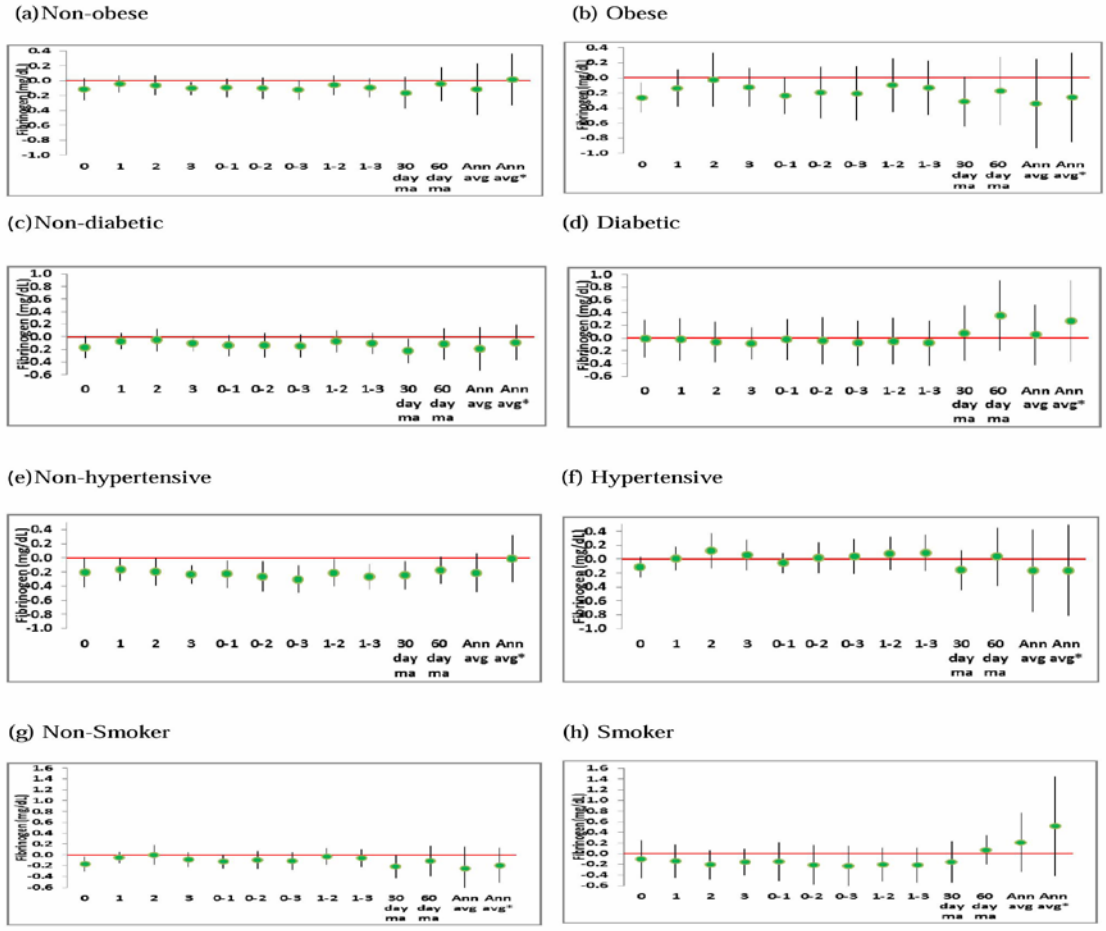

Figure 10. Effect Modification of associations between PM2.5 and Fibrinogen by disease condition at different lag times. Models are adjusted for age, gender, race/ethnicity, education, smoking status, body mass index, total cholesterol, HDL cholesterol, diabetes, hypertension, history of any cardiovascular diseases, maximum apparent temperature and ozone.

5.0 SYSTEMIC INFLAMMATORY RESPONSE TO PARTICULATE MATTER (PM2.5) AIR POLLUTION AND METABOLIC SYNDROME IN ADULT NHANES PARTICIPANTS

5.1 ABSTRACT

Background and Objectives: Previous studies suggest that individuals with preexisting cardio metabolic disorders may be at particularly increased risk of systemic inflammation by $PM_{2.5}$ air pollution, leading to increased risk of cardiovascular diseases. We investigated potential susceptibility of metabolic syndrome (MetS) participants to ambient $PM_{2.5}$ air pollution as suggested by increased markers of systemic inflammation compared to participants without MetS in adult National Health and Nutrition Examination Survey (NHANES) participants.

Methods: NHANES data (2001-08) on adult participants were merged with meteorological data from CDC WONDER and downscaler modelled air pollution data from the United States Environmental Protection Agency for each census tract in the 48 conterminous United States. The effects of short term (lags 0 to 3 and their averages), and long term (30 & 60 day moving average (ma) and annual average (anavg)) $PM_{2.5}$ levels on C-reactive protein (CRP, n=7134) and white blood cells count (WBC, n=7123) were analyzed using multiple linear regression, adjusting for age, gender, race, education, smoking status, history of any cardiovascular disease,

maximum apparent temperature and ozone, for participants with and without MetS. SAS SURVEYREG was used to account for the complex survey design of NHANES.

Results: : After adjusting for confounders, we generally observed an increase in CRP and WBC count with both short and long term ambient $PM_{2.5}$ air pollution exposure for participants with MetS compared to participants without MetS. For every 10 $\mu g/m^3$change in lag 0 $PM_{2.5}$, there was a significant positive change of 10.1% (95% CI: 2.2%, 18.6%) in CRP for participants with MetS, whereas for participants without MetS, change in CRP was - 1.3% (95% CI: 8.8 %, 6.8%). There were no significant associations for WBC count.

Conclusions: These data are consistent with the hypothesis that individuals with preexisting metabolic syndrome are susceptible to ambient $PM_{2.5}$ air pollution as expressed by serum markers of systemic inflammation. Further research is warranted to confirm these findings in large cohorts. With one third of the U.S. population compromised by MetS, the health impact of particulate air pollution in this sensitive population is likely to be significant.

5.2 INTRODUCTION

Metabolic syndrome (MetS), defined as a cluster of individual cardiovascular risk factors comprised of raised blood pressure, dyslipidemia (raised triglycerides and lowered high-density lipoprotein cholesterol), raised fasting glucose, and central obesity, increases the likelihood of cardiovascular disease (CVD) [104-106] and has been associated with systemic inflammation [107]. Studies have shown that MetS increases the risk of CVD to an extent greater than that conferred by any of its individual components [105, 108, 109]. Individuals with MetS have shown greater susceptibility to autonomic dysfunction (e.g. heart rate variability) in response to

$PM_{2.5}$ exposure [110]. Recently, a study reported higher risk of CVD mortality with increased levels of long term $PM_{2.5}$ exposure among participants with preexisting cardio-metabolic disorders e.g. diabetes, hypertension [111].

Previous studies have shown that the association between particulate matter (PM) exposure and systemic inflammation is stronger among participants with diabetes, obesity, and hypertension [19, 21, 30, 49]. These findings suggest that individuals with preexisting cardio metabolic disorders may be at particularly increased risk of systemic inflammation by PM air pollution. [107]. Therefore, it is important to study the effect of PM air pollution on systemic inflammation in participants with MetS. This is an important public health issue because of the enormous and growing prevalence of MetS worldwide, including one third population of the United States, and ubiquitous nature of air pollution.

Our objective is to use data from the National Health and Nutrition Examination Survey (NHANES), a US nationwide survey, to investigate potential susceptibility of MetS participants to $PM_{2.5}$ air pollution for markers of systemic inflammation, i.e., C-reactive protein (CRP) and white blood cell (WBC) count compared to participants without MetS. We hypothesized that participants with MetS will have increased response of change in biomarkers of cardiovascular risk to increase in $PM_{2.5}$ air pollution compared to participants without MetS.

5.3 MATERIALS AND METHODS

Health data. We used health data from the NHANES conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC) for the period 2001-08. Details about this survey and the specific measurement procedures and protocols have been

described on the NCHS website [92]. In brief, the NHANES followed a complex, stratified, and multistage probability sampling of the population of the US, with oversampling of minorities (African Americans and Mexican Americans) and the elderly (≥ 60 years of age). The survey consisted of an extensive household interview followed by a series of laboratory and other physical tests administered in a mobile examination center (MEC). Only those who completed the household interviews were invited for the MEC examination. Since 1999, the NHANES has been continuously conducted in two year cycles. The survey was approved by the Institutional Review Board of the NCHS, and informed consent was obtained before participation. The NHANES public use data sets were accessed for the four cycles of 2001-02, 2003-04, 2005-06, and 2007-08. We studied non-pregnant adults aged 20 and older having information available on all five criteria of MetS. After excluding pregnant women and participants with missing data on biomarkers levels and covariates of interest, there were and 7134 and 7123 participants included in our analysis for CRP and WBC, respectively.

Markers of Inflammation and covariates. The biomarkers of interest in this study were CRP and WBC count. In addition to these biomarkers, we also obtained data on demographic variables (including age, gender, race/ethnicity, and education) and potential risk factors for cardiovascular disease, such as smoking status and history of any cardiovascular diseases (i.e. congestive heart failure, coronary heart diseases, angina/angina pectoris, heart attack, stroke). Smoking status was defined as follows: current-presently smoking cigarettes or serum cotinine levels were greater than or equal to 10 ng/mL; former-have smoked 100 cigarettes in life but currently not smoking; never-had not smoked at least 100 cigarettes in life. We also obtained data from NHANES on infections within the last 30 days (cold, gastrointestinal illness, flu/pneumonia/ear infection), chronic obstructive pulmonary disease (COPD) (includes

emphysema and chronic bronchitis), household smoker presence and rheumatoid arthritis for conducting sensitivity analyses.

Definition of MetS. MetS was defined as per the Joint Scientific Statement Harmonizing the MetS [107]. According to these guidelines, MetS is defined as the presence of three or more of the following conditions: high blood pressure, hypertriglyceridemia, low high- density lipoprotein cholesterol (HDL-C), elevated fasting glucose, and abdominal obesity. High blood pressure was defined as systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg. For this analysis, high blood pressure could also be defined by a self-report of current use of antihypertensive medication. Hypertriglyceridemia was identified based on triglycerides ≥150 mg/dL. Low HDL-C was identified by HDL-C < 40 mg/dL in men or < 50 mg/dL in women. Elevated fasting glucose was defined as fasting glucose ≥100 mg/dL; for this analysis, elevated fasting glucose could also be defined by a self-report of current use of insulin or oral hypoglycemic. Abdominal obesity was defined based on waist circumference ≥ 102 cm in men and 88 cm in women. The details of the adult questionnaire, MEC examination, and laboratory tests for profiling MetS risk factors have been described on the NCHS website [92].

Ambient air pollution and weather data. Predictions of daily ambient 24-hour average $PM_{2.5}$ ($\mu g/m^3$) and 8-hour maximum O_3 levels (ppb) were obtained from the Environmental Protection Agency (EPA) using a downscaling modeling approach [93]. This downscaling approach uses Bayesian space-time modeling to combine air monitoring data and gridded numerical output from the Community Multi-Scale Air Quality Model (CMAQ) to produce point level daily air pollution predictions to the year 2000 US census tract centroids [94]. Daily predictions of O_3 and $PM_{2.5}$ were obtained from January 1, 2001 – December 31, 2008 at the

population weighted centroid (centers of population) of each year 2000 US census tract in the 48 conterminous states [95].

Meteorological data were obtained from the CDC WONDER North America Land Data Assimilation System Daily Air Temperatures and Heat Index (1979-2010) website [96]. Daily values of the maximum air temperature and maximum heat index for each county were extracted for the time period January 1, 2001 through December 31, 2008. Heat index incorporates both temperature and relative humidity and is a better measure on days when air temperature >80 F°. Maximum heat index was provided for those days when air temperature was above 80 F° or 26.7° C. CDC used a formula by Steadman to calculate the hourly heat index, from which the daily maximum heat index was computed [97]. For our analysis, we computed a daily maximum apparent air temperature which was defined as the daily maximum heat index if provided; otherwise the daily maximum air temperature was used.

We assembled an environmental database of daily pollution data and meteorological data for each census tract in the 48 conterminous United States for the time period January 1, 2001 through December 31, 2008. This large database contained predicted values of $PM_{2.5}$ and O_3 at the population weighted centroid of each year 2000 US census tract and maximum apparent temperature for each county assigned to the appropriate census tract level. In addition to the daily levels (lag 0), we calculated the following for $PM_{2.5}$ and O_3,: the level on the previous day (lag 1); two days prior (lag 2); three days prior (lag 3); the average of lags 0 and 1 (lag 0 to 1); average of lags 0, 1 and 2 (lag 0 to 2); average of lags 0, 1, 2, and 3 (lag 0 to 3); average of lags 1 and 2 (lag 1 to 2); and the average of lags 1, 2, and 3 (lag 1 to 3). The following long term averages were also calculated: the average of the 30 days prior (30-day moving average), 60 days prior (60-day moving average), and annual average.

Merging of health and environmental data. The Census tract (11 digit Federal Information Processing Standards code) of residence of each individual and the date of the NHANES examination were used to merge the NHANES data with the environmental dataset of air pollution and weather described above. Thus, each NHANES participant was assigned $PM_{2.5,}$ $O_{3,}$ and temperature exposure based on the census tract of residence.

Statistical analysis. We performed weighted descriptive analyses (mean and standard error) for each biomarker overall and also stratified by covariates. We examined exposure to ambient $PM_{2.5}$ as a predictor of each biomarker of interest- CRP and WBC in separate regression models. CRP was log transformed to improve normality and stabilize the variance. To evaluate the short term effects of $PM_{2.5}$ we analyzed the effect of $PM_{2.5}$ on the day of the blood draw (lag 0) as well on the day before (lag 1), two days before (lag 2), and three days before (lag 3) and averages of these time periods (lag 0 to 1, lag 0 to 2, lag 0 to 3, lag 1 to 2, and lag 1 to 3). In addition, we examined the long term effects of $PM_{2.5}$ on each biomarker by using the average $PM_{2.5}$ in the 30 days prior, 60 days prior and annual average value. We also examined the long term effects of $PM_{2.5}$ after adjusting for short term effects of air pollution (lag 0 to 3 of $PM_{2.5}$ and O_3).

We used multiple linear regression models to assess the association of $PM_{2.5}$ with each biomarker in subgroups: participants with and without MetS. The regression estimates were calculated for a 10 $\mu g/m^3$ increase in $PM_{2.5}$ after controlling for selected covariates based on prior biological and epidemiological knowledge of major determinants of cardiovascular health. Age was treated as a continuous variable, whereas gender, smoking (current smokers vs never & former smoker) and history of any cardiovascular disease were treated as dichotomous variables in the models. Race/ethnicity was categorized as non-Hispanic white, non-Hispanic black,

Mexican American, and other race. Education was categorized as 1-11th grade, high school grade/GED or some college, and college graduate. Models were run adjusted for the co-pollutant O_3 at the same lag or average as $PM_{2.5}$. Short term models were adjusted for maximum apparent temperature at the zero lag. The 30 day and 60 day moving average models were adjusted for 30 day maximum apparent temperature. The annual average models were not adjusted for temperature. Quartiles of temperature were used to account for non-linear relationship of temperature with biomarkers.

Sensitivity analyses. Certain medical conditions (e.g. rheumatoid arthritis and COPD), acute infections and presence of household smoking have been related to elevated levels of inflammatory markers [98-101]. Therefore, we investigated the sensitivity of our results to alternate ways of modelling by excluding people with history of (a) rheumatoid arthritis; (b) COPD; (c) acute infection in last 30 days; (d) household smoking. We also examined our results after controlling for season and year because temperature and pollutants show seasonal and yearly trend. All statistical analyses were performed using SAS software, version 9.2, Cary, NC, US. Descriptive analyses were conducted using PROC SURVEYMEANS and PROC UNIVARIATE.

All statistical analyses were performed using SAS software, version 9.2, Cary, NC, US. Descriptive analyses were conducted using PROC SURVEYMEANS and PROC UNIVARIATE. All regression models were run accounting for the complex sampling design of the NHANES with the SAS SURVEYREG command by using the sample weights included in the datasets. P-values <0.05 of were considered significant. All p-values were 2-tailed.

5.4 RESULTS

There were 7408 and 7397 non-pregnant adult participants with information available on all five criteria of MetS and CRP or WBC analysis respectively. Out of these, 35.1 % (N=2901 for CRP analysis group, N=2894 for WBC analysis group) were defined as having MetS as they met three or more criteria per the definition of MetS [107].

Table 8 shows the distribution of environmental variables from 2001 to 2008 at the level of participant's address on the day of blood draw. The mean ± standard error of $PM_{2.5}$ ($\mu g/m^3$), O_3 (ppb), and maximum apparent temperature (°C) were 11.74±0.37, 42.47±0.98, and 22.14±0.48, respectively.

Table 9 shows the survey weighted descriptive statistics of biomarkers for non-pregnant adult participants with information available on all five criteria of MetS, excluding participants with missing data on CRP and WBC levels and covariates of interest. The CRP levels were raised in black, older and male participants, whereas, WBC count were higher in younger, male and races other than black. The levels of both biomarkers were elevated in current smokers, lower education and presence of history of any CVD, rheumatoid arthritis, chronic obstructive pulmonary disease, and recent infections.

In single pollutant $PM_{2.5}$ models, there was an increased response of change in CRP in participants who had MetS compared to those who did not have MetS, for both short and long term exposure, after adjusting for age, gender, race, education, smoking status, history of any cardiovascular disease, and maximum apparent temperature. The increased response was greater for long term exposure. In bi-pollutant models (i.e., adjusting for ozone), there was a significant positive association of lag 0 $PM_{2.5}$ for participants with MetS. For every 10 $\mu g/m^3$change in $PM_{2.5}$, there was a change of 10.1% (2.2%-18.6%) for CRP. Also, for other lags, there was an

increase in CRP in participants who had MetS compared to those who did not have MetS (Figure 11).

Similar to CRP, there was an increase in WBC count in participants who had MetS compared to those who did not have MetS, for both short and long term exposure in single pollutant $PM_{2.5}$ models, after adjusting for age, gender, race, education, smoking status, history of any cardiovascular disease, and maximum apparent temperature. The increased response was more for long term exposure. In bi-pollutant models (i.e., adjusting for ozone), the point estimates were enhanced (Figure 12).

Sensitivity Analysis. The results of sensitivity analyses excluding people with history of (a) rheumatoid arthritis; (b) chronic obstructive pulmonary disease; (c) acute infection in last 30 days; (d) household smoking were largely similar. Additionally, adjusting for season and year led to similar results (Data not shown).

5.5 DISCUSSION

Our objective was to examine the association of $PM_{2.5}$ air pollution exposure with biomarkers, i.e., CRP and WBC, of cardiovascular risk in adult NHANES participants with MetS compared to participants without MetS. We found that participants with MetS had an increased response of change in biomarkers of cardiovascular risk to increase in $PM_{2.5}$ air pollution compared to participants without MetS.

Our study supports previous evidence that preexisting cardio metabolic diseases may confer susceptibility to particle-induced systemic inflammation. Previous epidemiological studies have found stronger effects of air pollution on inflammatory markers among diabetics

[21, 30, 49] and the obese [19]. Controlled human exposure studies in MetS participants [112] found significant positive associations of CRP to particle air pollution compared to studies in healthy adults [113-115].

However, there are a few studies that have examined the relationship of $PM_{2.5}$ with CRP and WBC count before in people with MetS. Dubowsky et al. 2006 found consistently significant positive associations for moving averages of 1-7 days of $PM_{2.5}$ exposure by presence of diabetes, obesity individually or diabetes, obesity and hypertension together in a panel study of 44 elderly participants for CRP [30]. In the same study, there was non-significant increased response for WBC count. Using the NHANES data, a study reported a significant incremental change in WBC count to long term exposure of PM_{10} according to a number of MetS criteria. However, they utilized NHANES III (1989-1994) participants who lived in urban areas, and the pollutant data from US EPA Air Quality System monitors PM_{10} levels during this earlier time period (1989-1994) were significantly higher (1 year average PM_{10} = 36.8 ±13 $\mu g/m^3$).

The stronger associations of $PM_{2.5}$ exposure with biomarkers of cardiovascular risk in MetS participants, who are already at higher risk of CVD, are likely due to already primed cellular machinery for the generation of excess reactive oxygen species and proinflammatory responses [116]. Animal studies have shown dysregulation of normal cardiac, vascular, and autonomic responses to inhalation exposure of O_3 and $PM_{2.5}$ in rats with high fructose diet induced MetS [117].

To our knowledge, this is the first nationwide population-based study examining the association of short and long-term exposure to $PM_{2.5}$ air pollution with inflammatory biomarkers of cardiovascular risk. The $PM_{2.5}$ exposure was assessed by using pollutant predictions at the population weighted centroid of the census tract using downscaling model approach from EPA

[93]. This approach allows use of health data for nearly the entire country instead of being limited to urban areas due to its ability to predict air pollutant concentrations for a large spatial extent and makes study findings generalizable to the US. Additionally, it better predicts temporal variability indicative of air pollutant concentrations measured at air quality monitors compared with earlier CMAQ models and spatial interpolation methods [93, 103]. Our study was able to consider health effects at the lower end of ambient particulate matter exposure compared to previous studies [54] . As a result, this investigation suggests that even at a low level of air pollution, those with multiple pre-existing cardiovascular risk factors might have an increased risk of cardiovascular disease when exposed to particulate matter air pollution.

This study must be interpreted in the context of its known limitations. There is a potential of exposure misclassification due to less confidence in the pollutant predictions in rural areas because of the increasing distance of these locations from air quality monitors [103]. Additionally, there is a possibility of error in exposure measurement due to use of average population exposure rather than individual exposure estimates and not accounting for the time spent indoors vs outdoors by the participants.

Conclusion

In summary, participants with MetS, compared to participants without MetS, showed a stronger positive response in systemic inflammation, as manifested by CRP and WBC count, in association with particulate air pollution (both short term and long term).With one third of the U.S. population meeting criteria for MetS, the health impact of particulate air pollutant in this sensitive population has the potential to be significant.

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5.6 TABLES AND FIGURES

Table 8. Distribution of environmental variables

Year	N	PM2.5(μg/m^3)	O^3 (ppb)	Temperature (° C)
2001	812	11.00±0.76	41.48±1.79	23.57±1.47
2002	922	12.60±1.66	47.00±3.54	23.27±1.27
2003	799	12.20±0.91	43.18±2.21	21.76±0.93
2004	859	11.38±0.92	38.93±1.72	22.39±1.59
2005	766	11.78±0.83	42.70±2.60	21.04±1.32
2006	832	10.81±0.73	39.56±1.69	21.25±1.09
2007	1054	12.80±1.61	44.35±3.25	21.78±1.97
2008	1090	11.51±0.75	43.03±2.19	22.22±1.20
2001-2008	7134	11.74±0.37	42.47±0.98	22.14±0.48

*Values are mean ± SE

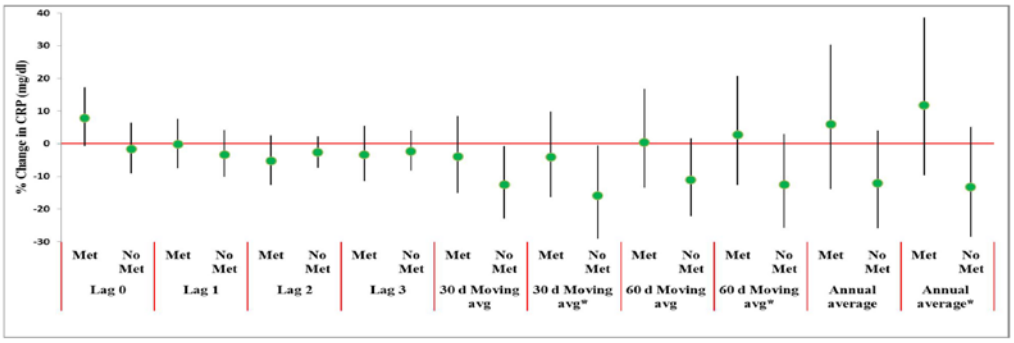
Table 9. Survey weighted descriptive statistics of biomarkers for non-pregnant adult participants with information available on all five criteria of metabolic syndrome

	C - reactive protein		White blood cells count	
	n (%)	Mean±SE (mg/dL)	n (%)	Mean ± SE (10^3 cells/µL)
Overall	7134(100)	0.4±0.01)	7123(100)	6.78± 0.04
Age (years)				
20-39	2302(38.6)	0.35±0.02	2299(38.5)	6.85±0.05
40-59	2321(39.3)	0.42±0.02	2318(39.3)	6.73±0.05
60+	2511(22.2)	0.46±0.02	2506(22.2	6.77±0.08
Gender				
Male	3651(49.6)	0.34±0.01	3645(49.7)	6.83±0.05
Female	3483(50.4)	0.47±0.01	3478(50.3)	6.74±0.05
Race/Ethnicity				
White	3722(72.4)	0.39±0.01	3717(72.4)	6.84±0.05
Black	1363(10.9)	0.50±0.02	1362(10.8)	6.25±0.06
Hispanic	1381(7.6)	0.41±0.02	1380(7.6)	6.85±0.07
Others	668(9.1)	0.39±0.04	664(9.1)	6.89±0.09
Education				
1-11th grade	1993(17.8)	0.44±0.02	1987(17.8)	7.12±0.08
HS grad/GED or some	3708(56.4)	0.43±0.02	3704(56.5)	6.87±0.05
college	1433(25.8)	0.32±0.02	1432(25.7)	6.37±0.05
College graduate				
Smoking				
Never	3653(51.1)	0.38±0.02	3645(51.1)	6.38±0.03
Former	1894(24.6)	0.41±0.02	1891(24.6)	6.56±0.07
Current	1587(24.3)	0.45±0.02	1587(24.4)	7.85±0.07
History of any CVD				
Yes	801(8.2)	0.56±0.05	798(8.2)	7.19±0.12
No	6333(91.9)	0.39±0.01	6325(91.9)	6.75±0.04
Recent Infection				
Yes	1904(27.1)	0.54±0.03	1903(27.1)	7.02±0.06
No	4981(72.9)	0.36±0.01	4972(72.9)	6.69±0.05
Rheumatoid Arthritis				
Yes	369(4.01)	0.57±0.04	367(4.0)	7.10±0.16
No	6754(96.0)	0.40±0.01	6745(96.0)	6.77±0.04
COPD				
Yes	538(7.4)	0.65±0.06	539(7.5)	7.27±0.11
No	6574(92.6)	0.38±0.01	6562(92.6)	6.74±0.04
Household Smoker				
Yes	1376(20.3)	0.47±0.03	1376(20.3)	7.67±0.08
No	5711(79.7)	0.39±0.01	5700(79.7)	6.56±0.03

Abbreviations: CVD, cardiovascular diseases

* excludes participants with missing data on CRP and WBC levels and covariates of interest

(a)



(b)

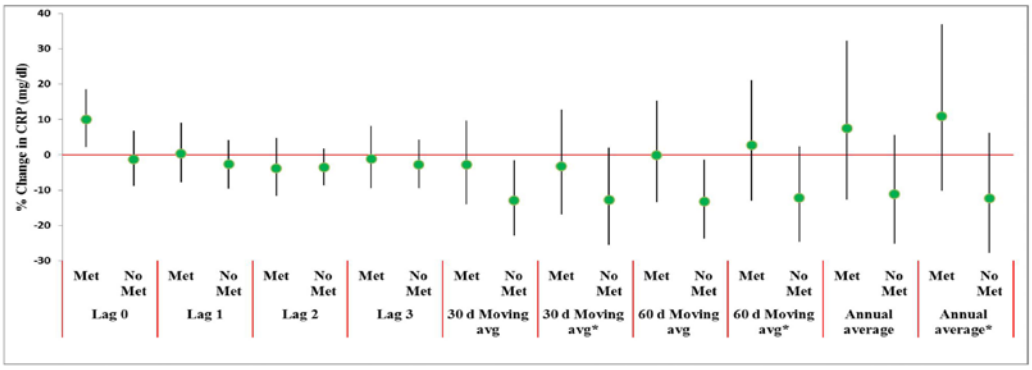
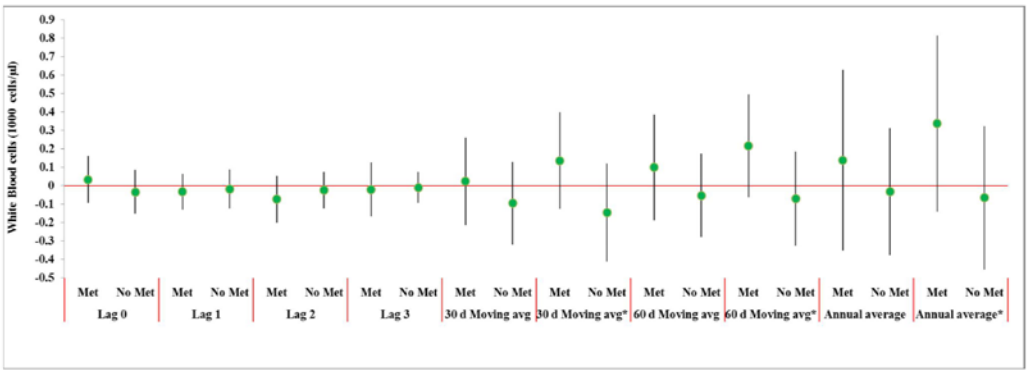


Figure 11. (a) Effect Modification of associations between PM2.5 and C - reactive protein (CRP) by metabolic syndrome. Models are adjusted for age, gender, race/ethnicity, education, smoking status, history of cardiovascular disease, and maximum apparent temperature. (b) Models were also adjusted for the same lag ozone.

(a)



(b)

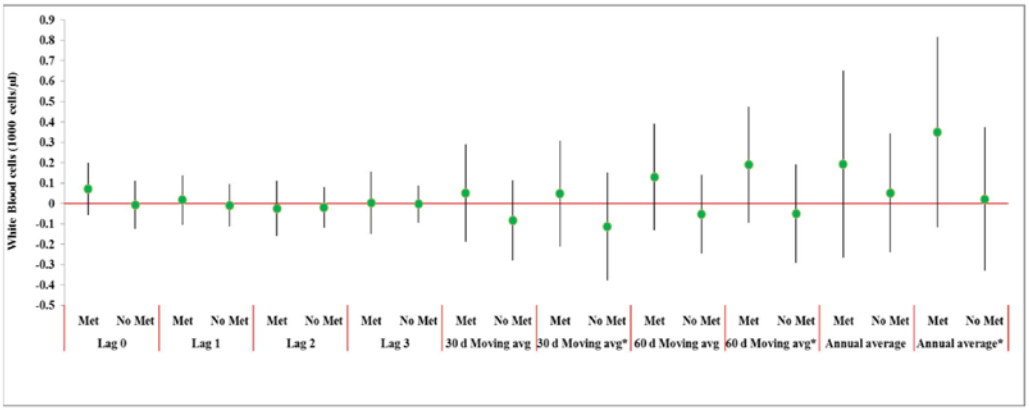


Figure 12. (a) Effect Modification of associations between PM2.5 and White Blood Cell (WBC) counts by metabolic syndrome. Models are adjusted for age, gender, race/ethnicity, education, smoking status, history of cardiovascular disease, and maximum apparent temperature. (b) Models were also adjusted for the same lag ozone.

6.0 SUMMARY AND PUBLIC HEALTH SIGNIFICANCE

Short term exposure to ambient $PM_{2.5}$ air pollution was significantly associated with CVDs mortality, specifically, IHD and PVD, in Allegheny County, PA. The risk of IHD mortality due to $PM_{2.5}$ air pollution was significantly greater for individuals who died outside of a hospital or nursing home compared to deaths in the hospital or nursing home. This could be due to exposure to high level of air pollution outside of hospital or nursing home, difficulty in accessing timely health care leading to significantly higher effect of air pollution on CVD/ IHD mortality or may be due to more accurate exposure assessment based on spatiotemporal kriging method at zip code of residence. This needs to be explored further. To understand the biological pathway linking $PM_{2.5}$ exposure with CVDs, a nationwide representative sample of adult men and women was utilized. Suggestive evidence of stronger associations of $PM_{2.5}$ with biomarkers of cardiovascular risk i.e. CRP, WBC count, homocysteine and fibrinogen, in participants with elements of MetS e.g. obesity, diabetes, hypertension and smokers were observed. Further investigation showed a stronger positive response in systemic inflammation, as manifested by CRP and WBC count in association with particulate air pollution (both short term and long term), in participants with MetS, compared to participants without MetS. With one third of the U.S. population meeting criteria for MetS, the health impact of particulate air pollutant in this sensitive population has the potential to be significant.

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