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Meta-analysis of the Association between Acute Particulate Matter Exposure and Cardiovascular Disease

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Abstract

Cardiovascular disease, which involves a wide range of conditions, including narrowed or blocked coronary arteries, has remained the leading cause of death in the United States for over 50 years. The majority of cardiovascular conditions are preventable, which can be identified through risk factors. However, maintaining healthy life choices can be difficult for most Americans, as the vast majority live in populated urban cities. Urban life can pose hazardous conditions to individuals, especially air pollution. Air pollution includes gaseous pollutants, such as carbon monoxide, nitrogen oxides, ozone and sulfur dioxide, and particulate matter. Of these pollutants, particulate matter has become a significant concern for cardiovascular research. Currently, most studies have focused on individual studies about the association between particulate matter exposure and risk of cardiovascular disease; thus, this study seeks to test multiple studies on particulate matter exposure and risk of cardiovascular disease by developing a different statistical conclusion through a meta-analysis.

A total of 16 case-crossover and time series studies were searched in order to investigate the association between particulate matter exposure (diameter < 2.5 μm [$PM_{2.5}$] or diameter <10 μm [PM_{10}]) and cardiovascular outcomes. Each study included adults ranging from 45 to 85 years of age, living in U.S. metropolitan areas. A random-effects model was used to derive the overall effect estimates. Statistical analysis was performed using RevMan software version 5.3. Data analysis was prepared by separating and analyzing all 16 studies into three groups by their effect estimate: Hazard Ratio, Risk Ratio, and Odds Ratio (HR, RR, and OR). Forest plots and funnel plots were constructed to determine summary effect estimates and publication biases, respectively. Heterogeneity (I^2) and overall effect (z-score) tests demonstrated that there was a

significant difference among studies used in all groups ($p < 0.05$). Using a different approach, this meta-analysis study provided further evidence that particulate matter exposure increases the risk of cardiovascular disease.

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List of Abbreviations

CVD	Cardiovascular Disease
PM	Particulate Matter
PM_{10}	Particulate Matter under 10 μm
$PM_{2.5}$	Particulate Matter under 2.5 μm
ROS	Reactive Oxygen Species
IL	Interleukin
HRV	Heart Rate Variability
SMD	Standard Mean Difference
HR	Hazard Ratio
OR	Odds Ratio
RR	Risk Ratio
SE	Standard Error
CI	Confidence Interval

Chapter 1. Introduction

Cardiovascular disease (CVD), the underlying cause of heart-related deaths in the U.S., claims more lives each year than all forms of cancer and chronic lower respiratory diseases combined. It has persisted for the past century as the most common type of death in the U.S. (1, 2). About 2,300 Americans die from some form of cardiovascular disease each day (2).

CVD involves a wide range of conditions that include narrowed or blocked coronary arteries that can lead to myocardial infarctions, angina, or a transient ischemic attack. However, a large proportion of CVD is caused by atherosclerosis. Atherosclerosis refers to the walls of arteries becoming thick and stiff due to the buildup of fatty deposits called plaques. Generally, plaques build up within the arteries and result in blood flow occlusion. If an occlusion occurs, several other heart-related pathologies ensue (3,4).

Occluded arteries have been linked to specific cardiac-related pathologies that affect the heart's physiological function. Ischemic heart disease, which involves narrowed coronary arteries, decreases blood flow to the myocardium. Additionally, the excessive force of blood pumping through the vasculature can lead to hypertensive-related diseases in response to arterial occlusion. On rare occasions, inflammation of the pericardium can occur. If the disease is left untreated, myocardial infarction, or in worst instances, a stroke may transpire (8-10).

Although suffering from CVD may be disheartening due to the intimidating statistics and consequences associated, it is preventable. Researchers have identified several risk factors that contribute to CVD. While identifying risk factors and promoting a healthy lifestyle is attainable, individuals, especially in urban-living societies, might experience a difficult time adjusting. Current urban conditions can pose a dangerous health hazard to the population, including inadequate water supply, inadequate energy access, and increased pollution exposure. Pollution, notably particulate matter, can pose the most significant health risk as researchers previously associated particulate matter exposure to increases in chronic respiratory and cardiovascular diseases (29). Classified by their diameters, PM_{10}, $PM_{2.5}$, and ultrafine particulate matter have been found significantly associated with cardiovascular mortality (30-32). Additionally, chronic particulate matter exposure is associated with increased oxidative stress markers (42-43) and advanced atherosclerosis (41,46).

While many individual studies have investigated the association between particulate matter exposure and CVD development, there are a few shortcomings. Disadvantages can include the validity of a hypothesis based on a single study's results and subjective narrative views. Combining multiple studies into a simplified statistical procedure can provide deeper insights into the original research question.

Purpose

The purpose of this study is to determine how consistent exposure to particulate matter increases the risk of CVD in each study by performing a meta-analysis to identify the common effect.

Scope

Cardiovascular disease includes several other heart-related conditions such as myocardial infarction, angina, stroke, and atherosclerosis potentially exacerbated by air pollutants. We will determine the extent of particulate matter exposure and risk of CVD development by analyzing several relevant studies with various study designs and effect estimates, including hazard, risk, and odds ratios.

Assumption

1. Cardiovascular disease is worsened with exposure to particulate matter, acutely, and chronically.

Hypothesis

Hypothesis 1: Particulate matter exposure increases the risk of developing cardiovascular disease.

Significance

For the past century, cardiovascular disease has persisted as the most common cause of death in the U.S. Additionally, acute and chronic exposure to particulate matter has increased the risk of chronic respiratory and cardiovascular diseases. Using a meta-analysis approach by combining several other primary authors' findings to create a new statistical analysis will provide additional evidence of the association between exposure to particulate matter and cardiovascular disease.

Chapter 2. Review of Literature

Cardiovascular disease

CVD is the leading cause of death for adults among most racial and ethnic groups in the United States. Researchers have reported that 647,000 Americans die from some form of cardiovascular disease each year, which is 1 in every 4 deaths (1). Cardiovascular disease costs the United States about $219 billion each year (2). These costs include health care services, medicines, and lost productivity due to death (1).

CVD refers to a group of chronic disorders of the heart and blood vessels that become occluded or narrowed over time. The most common cause is a buildup of fatty deposits called plaques, located on the blood vessels' inner walls that supply the heart or brain. Plaque is comprised of fat, cholesterol, calcium, waste products from cells and fibrin, a clotting agent. Over time, plaque hardens and narrows the arteries, limiting the flow of oxygen-rich blood to organs and other parts of the body called atherosclerosis. Heart attacks and strokes are generally acute events caused by a blockage that prevents blood from flowing to the heart or brain.

In addition, atherosclerosis can occur systemically in the body; for example, in arteries located in the heart, it is known as coronary artery disease, and in the legs, peripheral arterial disease. Gradually, plaques build up within the arteries and result in blood flow occlusion; if this occlusion occurs in the coronary circulation, it serves as a direct cause of cardiac pathologies. Cardiac pathologies, such as those that affect the heart's anatomical structure and its physiological function, include ischemic heart disease, hypertensive heart disease, and inflammatory heart disease (3,4).

Ischemic heart disease, also named coronary heart disease, is the most common type of cardiovascular disease, killing over 370,000 Americans each year (5). Ischemic heart disease accounts for 46% and 38% of deaths in males and females, respectively (6,7). Ischemic heart disease is the narrowing or blockage of the coronary arteries, which supply blood and oxygen to the myocardium. Further impairment can lead to angina or myocardial infarction.

Angina manifests as pain in the chest and results from reduced blood supply to the heart. Usually, patients with ischemic heart disease experience angina; however, individuals with valvular disease, hypertrophic cardiomyopathy, and uncontrolled hypertension may also experience angina (8). On rare occasions, patients with normal coronary arteries may experience angina related to coronary spasms or endothelial dysfunction (9,10). Myocardial infarction may also occur when a portion of the heart is deprived of oxygen due to the blockage of coronary arteries. Nationally, existing cases for myocardial infarction are estimated at 9.2 million in adults older than 20 years of age and have claimed approximately 117,000 lives in 2018 (1). When coronary arteries cannot adequately supply oxygenated blood to myocardial cells, some of them die, creating the infarction.

Hypertensive heart disease, which accounts for 6% of cardiovascular deaths in the U.S., is generally applied to various heart diseases, such as hypertension, and heart failure, caused by direct or indirect effects of elevated blood pressure (8,10). These diseases generally develop in response to excessive force of blood pumping through blood vessels; however, acute elevation of blood pressure can exacerbate specific symptoms associated with chronic hypertension, such as cardiac arrhythmia (11). Inflammatory heart disease, also known as myocarditis, a rare viral

infection to the myocardium, accounts for 2% of cardiovascular deaths in the U.S. (12). Specific myocarditis conditions include cardiomyopathy, a genetic or infection-based condition, and pericardial disease (4). Pericardial disease refers to the inflammation, fluid accumulation or stiffness of the sac that encases the heart, called the pericardium (13).

Risk Factors

There are many particular behaviors and conditions that may increase a person's risk for developing CVD. Some risk factors, such as family history, cannot be modified by treatment, yet high blood pressure, can be prevented. While risk factors are associated with acute events (e.g., myocardial infarction/stroke) and chronic conditions (e.g., CVD), the disease's progression is largely dependent on lifestyle. Acquiring more risk factors will increase the likelihood of developing CVD.

Identifying individuals at higher risk for cardiovascular events is an essential resource that can reduce the disease's burden on individuals and society. The Framingham Heart Study has appropriately highlighted each established risk factor for CVD and integrated them into risk scores, which provides a quantitative prediction on an individual's future risk for CVD (14). The following risk factors include tobacco smoking (15), physical inactivity (16), poor nutrition (17), overweight/obesity (18), high cholesterol (19), high blood pressure, and diabetes mellitus (20,21). Worldwide, smoking tobacco was the second leading risk factor for disease and contributed to an estimated 7.2 million deaths in 2015 (15). Additionally, more than 480,000 Americans have died from tobacco-related diseases (15).

Physical activity at any age protects against a vast majority of chronic health problems, including CVD. Studies have shown that physical activity protects the body by regulating weight and improving its insulin use. Furthermore, physical activity can reduce blood pressure, blood lipid levels, blood glucose levels, and blood clotting factors. Approximately 30.4% of U.S. adults do not engage in physical activity. Although a healthier diet has slightly improved over the past decade, overweight/obesity has steadily increased in the U.S. Nationwide, the prevalence of obesity among adults increases from 1999 to 2000 through 2013-2014 from 30.5% to 37.7% (18). The intake of dietary fats is an essential nutritional factor that affects total cholesterol concentration. Approximately 40% of American adults have total cholesterol levels above 200 mg/dL, considered borderline hyperlipidemia (19). Approximately 12% of Americans have cholesterol levels above 240 mg/dL, which is considered high.

Hypertension is a prevalent condition worldwide and a significant risk factor for CVD. According to the American College of Cardiology/American Heart Association (ACC/AHA) Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults, approximately 46% of U.S. adults have hypertension (20). Reports from 2015 indicated that there were 79,000 deaths primarily attributed to high blood pressure (20). Diabetes mellitus, a significant risk factor for CVD, is the 7th leading cause of death in the U.S (21). More Americans die each year from diabetes than AIDS and breast cancer combined. An estimated 9% of U.S adults have been diagnosed with diabetes mellitus (22), in contrast to 3% of U.S adults who have undiagnosed or untreated diabetes. Additionally, 34% of adults have prediabetes (23).

Although making preventative measures to decrease risk factors against CVD is attainable, there are several underlying determinants of CVD. These reflect of the major forces driving social, economic, and cultural changes, such as globalization, urbanization, and population aging. The population density in urban U.S. cities is more than 46 times higher than that of the territory outside cities (24). The average population density for cities is approximately 1593.5 people per square mile than rural cities, approximately 34.6 people per square mile (25). Urbanization offers numerous opportunities for individuals; however, as urban life brings new opportunities, it also creates unique health challenges. There has been extensive research investigating a link between city living, and adverse health outcomes from researchers focused on environmental health, public health, and behavioral health (26–28). While urban living continues to offer many opportunities, including potential access to better healthcare, today's urban environments can also lead to health risks and introduce new hazards. Hazards that particularly relate to city life include inadequate water supply and energy access, and increased pollution exposure. Pollution, in particular, promotes the most dangerous effect on human health.

Particulate Matter

Air pollution has emerged as a leading problem for environmental health in the world. It can be potentially detrimental to an individual's health and result in chronic respiratory diseases and CVDs. Numerous studies demonstrate a strong association between air pollution exposure and increased morbidity and mortality (29–31). Air pollution includes gaseous pollutants, such as carbon monoxide, nitrogen oxides, ozone and sulfur dioxide, and particulate matter (29). Of these pollutants, ambient particulate matter has become a significant concern for cardiologists and specialists in environmental medicine.

Ambient particulate matter is defined as the material suspended in the air as minute solid particles or liquid droplets, derived from both human and natural activities (32). Ambient particulate matter is a heterogeneous mixture with varying size and chemical composition (30,32). Generally, ambient particles include inorganic components, such as sulfates, nitrates, ammonium, chloride and trace metals, elemental and organic carbon, crystal materials, biological components such as bacteria, spores, and pollens, and adsorbed volatile and semi-volatile organic components (29,32). According to their diameter, they are classified as PM_{10}, $PM_{2.5}$ and ultrafine particles in reference to their potential influence on human health. PM_{10} has a diameter from 2.5 to 10 μm. These particles usually come from road and agricultural dust, tire wear emission, construction and demolition work, or mining operations. In addition, natural activities such as wildfires and windblown dust are sources of PM_{10}. Compared to PM_{10}, the primary contributors of $PM_{2.5}$ are from traffic and industry that includes fuel combustion from power plants, oil refineries, or brake emissions from automobiles. $PM_{2.5}$ are particles that are less than 2.5 μm. Established from observational and epidemiological studies, $PM_{2.5}$ is generally

associated with increased risks of myocardial infarction, arrhythmia, and heart failure (30–33). Diameters of less than 0.1μm include ultrafine particles, which are primary sources of tailpipe emissions from automobiles.

Table 1: PM classification. Table 1 displays the three types of Particulate Matter with their corresponding description.

PM Size	Description
PM_{10} (2.5 to 10 micrometers)	Road and agricultural dust, tire wear emission, construction, demolition work, mining operations, wildfires, windblown dust
$PM_{2.5}$ (<2.5 micrometers)	Fuel combustion from power plants, oil refineries, brake emissions from automobiles
Ultrafine Particles (< 0.1 micrometers)	Tailpipe emissions from automobiles

Effects of particulate matter on the respiratory system

Theoretically, PM_{10} particles localize in the upper airways, while $PM_{2.5}$ and ultrafine particles spread to the bronchioles and alveoli. Ultrafine particles and $PM_{2.5}$ further penetrate the alveolar-capillary membrane, eventually spreading into the pulmonary circulation before entering the systemic circulation (32,33). Evidence has shown that ultrafine particles can be found in remote organs such as the heart and lungs and induce specific toxic effects (34,35). Associations between $PM_{2.5}$ and ultrafine particles is linked to endothelial dysfunction (36,37), vasoconstriction (38), increased blood pressure (39), prothrombotic and coagulant changes (40,41), systemic inflammatory and oxidative stress responses (42,43), autonomic imbalance and arrhythmias (44,45), and progression of atherosclerosis (41,46).

The consensus is that once deposited in the lungs; particulate matter triggers an inflammation-related cascade (35,47). Inflammation is a vital response to injury and stimulates healthy tissue regeneration; however, an excessive inflammatory response is harmful and can lead to disease. Short-term exposure to particulate matter has an acute inflammatory effect on human airways. Notably found in $PM_{2.5}$ and ultrafine particles, short-term exposure results in mast cell and neutrophil activation. Immune cell activation results in cytokine and chemokine production (40,42). Recruitment of inflammatory cells from the circulation into the airway mucosa during an inflammatory response involves a series of coordinated events. These include recruitment of leukocytes from the blood to the luminal surface of postcapillary venules, adhesion to endothelial cells via induced upregulation of adhesion molecules, trans-endothelial migration, and movement along a chemotactic gradient toward the site of inflammation. Pulmonary inflammation induced by ultrafine particles can also trigger a ROS-dependent response (48). ROS, or reactive oxygen species, is a group of free radicals associated with atherosclerosis and vascular dysfunction (43,46,49,50).

Mechanisms of particulate matter on the cardiovascular system

Nevertheless, researchers have collectively agreed upon three mechanisms that influence the cardiovascular system: inflammation, translocation of particles, and an imbalance in the autonomic nervous system.

Inflammation

Inhaling PM, specifically ultrafine particulate matter, may provoke a low-grade pulmonary inflammatory response. An inflammatory response can release harmful cytokines that change blood coagulability (40) and trigger other related physiological responses, including acute cardiovascular events and quicker development of atherosclerosis and CVD. Experimental studies on particulate matter exposure chambers revealed that acute exposure increased inflammatory activity in the airways, and peripheral blood, eliciting endothelial dysfunction (38,50). Additional studies demonstrated systemic inflammation and progressive atherosclerotic plague damage from acute and chronic exposures to particulate matter (51–53). Short term exposure to particulate matter is associated with increased levels of C-reactive protein (54,55), interleukin-6 (56), fibrinogen (57), plasma viscosity (55), soluble intercellular adhesion molecule 1 (58,59), vascular cell adhesion molecule 1 (58,60), subclinical pulmonary inflammation and markers of oxidative stress (61). In comparison, chronic exposure increases carotid intima-media thickness (62), fibrinogen production (55), platelet (63), and white blood cell count (64). Elevated levels of both inflammatory and oxidative stress markers due to PM exposure increase circulation within the coronary vessels, contributing to arterial hypertension, inflammation, progression of myocardial ischemia, and atherosclerosis development.

Oxidative stress pathways indirectly influence vascular damage through particulate matter. Increases in oxidative stress and inflammatory reactions are in response to $PM_{2.5}$ or ultrafine particle exposure (42,65). Human trial studies discovered that $PM_{2.5}$ and ultrafine particle exposure increased circulating levels of pro-inflammatory cytokines such as C-reactive protein, a biomarker of systemic inflammation, IL-6, IL-8 and IL-1beta (66,67). Increased levels

of inflammatory markers yielded an increase in blood coagulability, causing endothelial dysfunction and acute vasoconstriction (38,40,68).

In conjunction with previous mechanisms concerned with particulate matter and the circulatory system, a ROS-dependent mechanism in the myocardium and endothelial cells is similarly responsible for toxic effects in the cardiovascular system. Increased ROS amounts were associated with myocardial stunning, necrosis, vascular dysfunction, and apoptosis (43,48,49). Myocardial stunning is a segmental wall-motion abnormality in which ventricular dysfunction persists despite restoration of normal blood flow. ROS has been linked with arrhythmias among patients undergoing coronary artery bypass surgery. Additionally, ROS production contributes to the pathogenesis of heart failure (49,69).

Translocation

Although the actual mechanism of particulate matter translocating to the blood is not well understood, there is consensus about inhaled ultrafine particles translocating into circulation. Translocation into the bloodstream appears to go through the gap-fenestration pathway, which travels to the air-blood barrier and adheres to red blood cells (70). Key steps include forming a large-sized gap between type I alveolar epithelial cells, passive transfer into the basement membrane, and translocation into the capillary lumen with the use of endothelial fenestration or transcytosis (71). These events are followed by systemic circulation after ultrafine particles adhere to the red blood cells' cell membrane. Studies have shown that increased ultrafine particle exposure is associated with increased toxicity within cells, ultimately interacting between intracellular proteins and organelles (72,73).

Particulate matter translocated into the systemic circulation can negatively impact vascular function by stimulating atherosclerosis and thrombosis. Translocation has been associated with arterial vasoconstriction by disrupting endothelial vasodilation and endogenous fibrinolytic activity through ROS (73). Furthermore, particulate matter inhibits nitric oxide synthase activity (74), which prevents the release of nitric oxide into the bloodstream, causing vasoconstriction.

Autonomic nervous system imbalance

In addition to the evidence presented for inflammation and translocation as a pathway of particulate matter affecting the heart, several studies have shown acute responses involving heart rate changes and blood pressure. These variables have suggested an association with increased particulate matter levels, proposing a possible effect on the autonomic nervous system. Recently, evidence suggested that increases in heart rate are more susceptible to individuals pre-disposed with high blood viscosity (75,76). Heart rate variability (HRV) is a measure of cardiac autonomic function, as cardiac output varies in response to signals from various parts of the autonomic nervous system. Decreases in HRV are strong predictors of mortality and CVD (77,78) . Controlled experiments and observational studies examining HRV and exposure to particulate matter demonstrated an association between increased heart rate and decreased HRV (79,80) and a reduced parasympathetic tone (79). Increased levels of PM_{10} levels significantly raised the odds of an increase in heart rate by 5 to 10 beats per minute (39), while several experimental studies have indicated $PM_{2.5}$ as a contributor to a change in blood pressure (81,82). Exposure of at least 2 hours to $PM_{2.5}$ can increase systolic and diastolic blood pressure in

normotensive patients, while patients undergoing 120 hours of $PM_{2.5}$ exposure had increased resting systolic and diastolic pressures (83,84).

Applying a meta-analysis approach to particulate matter exposure and cardiovascular disease has the potential to provide further insight and validity to previous research. While various studies suggest consistency between PM exposure and CVD rates, a meta-analysis can be used in this condition to identify the common effect. The study aims to investigate the hypothesis that particulate matter exposure will increase the risk of developing CVD.

Chapter 3: Methodology

Study Criteria

Both case-crossover and time-series studies investigating the association between particulate matter exposure and cardiovascular heart disease outcomes in the United States were identified. Additionally, minimum and intermediate outcomes of cardiovascular disease were identified. Studies were retrieved by searching free text and keywords in Pubmed. Search terms for particulate matter exposures included "particulate matter" as well as particulate matter with diameters less than 10 μm, or 2.5 μm. The literature search was restricted to articles published in major U.S. cities from January 1st, 2006 through June 2020.

Definition of Outcomes

In order to methodically review the data presented, studies included adults (men and women) ranging from 45 to 85 years of age, living in U.S. metropolitan areas. Studies measured participants' variables at baseline to assess various individual risk factors. These included age, gender, smoking status, body mass index, congestive heart failure, hypertension, hyperlipidemia, diabetes, family history of early coronary artery disease, and a total number of diseased coronary vessels. Smoking included active or previous (greater than 10 pack-years) tobacco use, body mass index was calculated from height and weight. Congestive heart failure was reported based on clinical symptoms. Hypertension was reported for systolic blood pressure higher than 140 mm Hg, diastolic blood pressure greater than 90 mm Hg, or use of anti-hypertensive drugs. Hyperlipidemia was physician reported, and blood sample reported for total cholesterol greater than 200 mg/dL, low-density lipoprotein level greater than 130 mg/dL, or use of cholesterol-

lowering medication. Diabetes was determined based on physician-reported fasting blood glucose level greater than or equal to 126 mg/dL or use of an anti-diabetic medication. Family history was based on a self-reported survey, which asked if there were any first-order relatives that suffered from myocardial infarction, coronary revascularization, before the age of 65. The number of severely diseased coronary vessels were defined as 0, 1, 2, or 3 coronary arteries with greater than or equal to 70% maximal stenosis, which was determined via coronary angiography. Studies that involved patients who presented with one of three general clinical conditions of ischemic heart disease were included in the study. General clinical conditions included acute myocardial infarction, stable angina, or stable noncoronary syndromes. In contrast, patients presented with physician-diagnosed ischemic heart disease or equivalent before particulate exposure (i.e., carotid artery disease, peripheral arterial disease) were excluded. Studies were included if they presented original data for particulate ($PM_{2.5}$ or PM_{10}) air matter and reported hospitalization or mortality due to cardiovascular heart disease.

To evaluate immediate and delayed associations, time-series and case-crossover studies were used to analyze the associations between particulate exposure and cardiovascular heart disease while taking into account different lag patterns, using either single-day lags (current day concentration), lag_7 (7 days before the event day), or cumulative lags (mean between the same day and the previous day). If several lag estimates were reported in the same article, the most frequently used leg estimate was chosen. Most studies have verified the linear assumption concerning the association between particulate air matter increase and cardiovascular disease risk.

Data Extraction

A total of 16 studies were used in the data analysis. For each study that met the inclusion criteria, information was extracted based on the study characteristics (authors, year of publication, U.S. city, study design), population characteristics (inclusion criteria, age), exposure assessment, outcome, and measures of association. Measures of association extracted from the published data were hazard ratio, odds ratio, and relative risk ratios. Where there were more than one analytical comparison group, both measures of association were extracted. For mortality studies, data were extracted for the major categories of overall CVD when presented.

Table 2: Details on included studies. Table 2 shows all 16 studies included in the meta-analysis. Each study lists the author and year, study design (e.g., time-series or case-crossover), effect estimate (e.g., HR, RR, or OR), as well as its effect size.

Study and Year	*Study Design*	*Effect Estimate*	*Effect Size*
Lipsett et al., 2010[85]	Time-series	Hazard ratio	124,614
Miller et al., 2007[86]	Time-series	Hazard ratio	58,610
Pope, 2015[81]	Time-series	Hazard ratio	669,046
Puett et al., 2009[87]	Time-series	Hazard ratio	66,250
Puett et al., 2011[82]	Time-series	Hazard ratio	17,545
Wichenthal, 2014[88]	Time-series	Hazard ratio	83,378
Chen et al., 2005[89]	Case-crossover	Risk ratio	3,329
Gan et al., 2011[90]	Case-crossover	Risk ratio	452,735
Lee et al., 2016[91]	Case-crossover	Risk ratio	236,551
Pope et al., 2004[92]	Case-crossover	Risk ratio	319,000
Pope et al., 2006[84]	Case-crossover	Risk ratio	12,865
Balluz et al., 2007[93]	Case-crossover	Odds ratio	15,968
Johnson et al., 2009[94]	Case-crossover	Odds ratio	132,224
Madrigano et al., 2013[95]	Case-crossover	Odds ratio	13,539
McGuinn et al., 2016[96]	Case-crossover	Odds ratio	5,679
Peel et al., 2007[97]	Case-crossover	Odds ratio	4,0000,000

A random-effects model was used to derive HR, RR, OR, as well as 95% CI. This model was chosen due to its ability of combining studies, which allows for any heterogeneity across studies. The random-effects model is the most conservative approach in this setting because it incorporates within and between-study heterogeneity in the confidence interval. Forest plots were implemented to determine the summary effect estimate, followed by funnel plots, which assessed publication bias.

Statistical analysis

Data were analyzed using RevMan software version 5.3. Outcomes were first collectively analyzed by the standardized mean difference (SMD). SMD, which assessed individual effect size, each study's 95% confidence interval, and its standard error. Additionally, SMD expresses the intervention effect size in each study relative to the variability observed in that study. SMDs lower than zero indicate the degree to which particulate matter exposure was associated with the risk of developing CVD.

Studies were subsequently separated and analyzed in three groups: Hazard Ratio (HR), Odds Ratio (OR), and Risk Ratio (RR). Separate analyses were necessary due to their specific measurements of association. Analysis was performed using a generic inverse variance data type for each group, followed by the inverse variance statistical method (98). RevMan calculated and provided a logarithmic value for each study, its corresponding weight, and a weighted effect estimate with its 95% confidence interval. Standard error (SE) was manually calculated from each study by subtracting the upper limit from the lower limit of the 95% confidence interval, dividing the whole number by 3.92 (99). However, for a ratio measure (e.g., RR, OR, or HR),

upper and lower limits were performed on a natural logarithmic scale. Below were formulas used to derive the upper/lower limits as well as the standard error.

Lower limit = ln(lower confidence interval given for RR, OR, HR)

Upper limit = ln(upper confidence interval given for RR, OR, HR)

SE = (upper limit – lower limit)/3.92

As standard error increases (i.e., the means are more spread out), it becomes more likely that any given mean is an inaccurate representation of the true population mean. Standard error increases when the variance of the population or the standard deviation increases, while standard error decreases when the sample size increases and the variance decreases.

Forest plots were used to determine how the effect estimates of each individual study are distributed around a null value and the overall effect estimates. Inspection involves all the included studies in the meta-analysis. The effect estimate of each study is presented in the form of a red square box. Across each study, the estimate runs a horizontal line; this length of the line represents the 95% confidence interval's width for the effect estimate for each particular study. The x-axis represents the weighted measurement of association (e.g., HR, RR, or OR). A vertical broken line passing through the neutral point indicates if one side favors intervention or favors the control. In addition to these two indications, there will also be two diamonds. These diamonds represent the summary effect estimate in the form of random effects meta-analysis.

Publication bias tests if any biases that can impact the study conclusions. In other words, a publication bias tests whether this meta-analysis omitted any studies that should have been included. Publication bias can occur from journal editors selecting studies with interesting findings or support studies with extensive, positive findings. In order to combat these issues, a funnel plot was assessed. Funnel plots tested the effect estimates of the studies on the x-axis and either the study's sample size or the effect measure variability, which is either the variance or standard deviation, on the y-axis of the plot. If publication bias does not occur, the plot would resemble a funnel with one or two dots representing studies with large sample sizes or low variance and an effect estimate close to or identical to the summary estimate. The base of the funnel will be populated by smaller sized studies or studies with larger variances. However, if there is publication bias, then it is expected that one of the quadrants of the funnel on the lower side will be absent or blank.

After examining the studies' heterogeneity, estimating the summary effect size, plotting the forest plot, and testing for publication bias, it is feasible to comment about the association between exposure and the outcome. However, there is still a possibility that certain aspects of the study need to be examined, or some characteristics of the participants need to be examined separately or in a separate analysis.

Chapter 4. Results

To assess summary effect estimates, a forest plot was analyzed for all groups. HR, OR, and RR were significantly different ($p < 0.05$) across study designs. OR group displayed 79% heterogeneity. In comparison, the HR group had the lowest heterogeneity I^2 test of 54%, suggesting the possibility of moderate heterogeneity. Additionally, the RR group had the highest I^2 test of 92%, indicating substantial heterogeneity across the data.

SMD plot indicated positive association between particulate matter exposure and development of CVD according to figures 1 and 5. 63% of the included studies displayed negative values, four studies each from OR and HR, while two studies came from RR. Although overall effect was not statistically significant while testing for heterogeneity, SMD plot was statistically significant ($p < 0.0001$), with an I^2 test of 82%, indicating substantial heterogeneity across the data sample.

While assessing heterogeneity, tau-squared (Tau^2), and a chi-squared (Chi^2) were also presented. Tau^2 represents the absolute value of the true variance, while Chi^2 is used to estimate the variance and the standard deviation of the true effects. HR had the smallest variation compared to the RR and OR. In contrast, RR had the greatest variation. Excess variation is noted between HR and RR, as there is a higher proportion of variation in RR ($Tau^2 = 0.01$, $Chi^2 = 47.38$) than HR ($Tau^2 = 0.00$, $Chi^2 = 10.80$). According to figures 2 and 4, OR had greater variation ($Tau^2 = 0.01$, $Chi^2 = 18.99$) than HR.

Total weighted averages were computed along with averaged 95% confidence intervals for each effect estimate. All effect estimates totaled an averaged ratio of above 1, indicating a positive correlation between exposure to $PM_{2.5}$ and cardiovascular disease (HR = 1.11, RR = 1.19, OR = 1.11). SE was determined from the natural log of each studies' 95% confidence intervals. Large SE's varied across each effect estimate, with one in RR (0.1298), and two in HR (0.1051, 0.164). The remaining SE's were smaller, which occurs when the sample size increases. An increase in sample size correlates to an accurate representation of the true population.

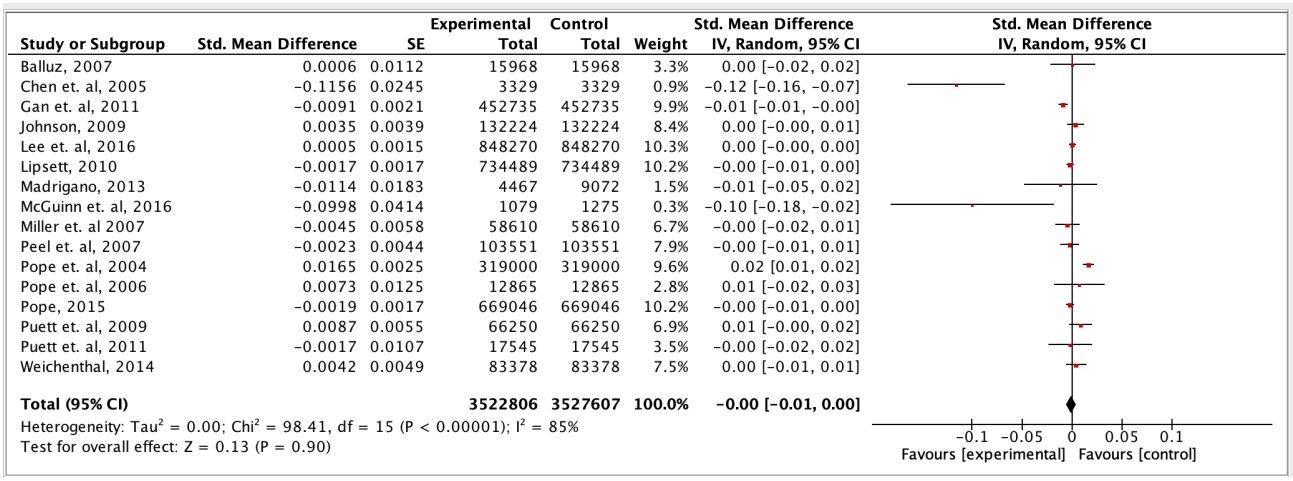

Figure 1. Standardized mean difference summary estimate and forest plot. Figure 1 shows a SMD summary estimate and forest plot. A total of 16 studies were analyzed, which provided their SMD, SE, weight in percent, and weighted SMD average along with its 95% CI. Additionally, each study included the total number of participants in the experimental group and the control group. The experimental groups were participants exposed to particulate matter and developed CVD. Heterogeneity and the overall effect of the analysis were assessed. To the right of the figure indicates the forest plot analysis. Longer horizontal lines indicated a more comprehensive 95% CI range. Smaller red boxes corresponded to smaller effect sizes, while larger red boxes described larger sample sizes. The black diamond box described the total estimate of the analysis.

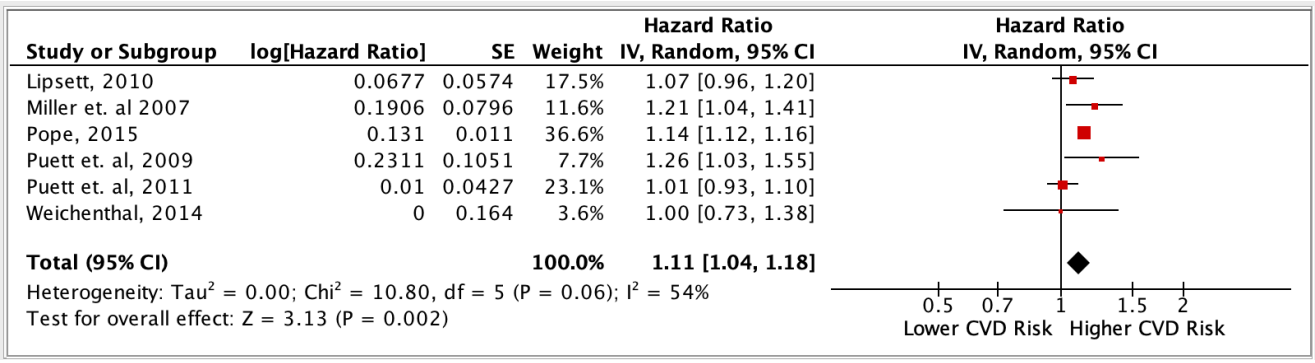

Figure 1. Hazard Ratio summary estimate and forest plot. Figure 2 displays the summary estimate and forest plot analysis for the HR group. Each individual study was analyzed by calculating their transformed HR, SE, weight, as well as its total HR and 95% CI. Additionally, total HR estimate and 95% CI (1.11 [1.04, 1.18]) were calculated. Heterogeneity and the overall effect of the analysis were assessed. To the right of the figure indicates the forest plot analysis. The larger red boxes indicated a larger effect size (Pope, 2015), while a longer horizontal bar running through the red boxes indicated a wider confidence interval. The black diamond box described the total estimate of the analysis.

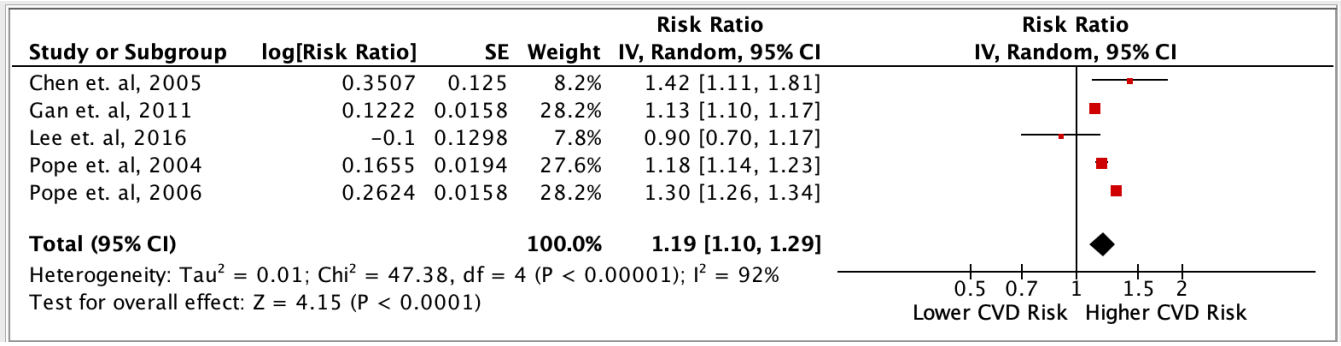

Figure 2. Risk Ratio summary estimate and forest plot. Figure 3 displays the summary estimate and forest plot analysis for the RR group. Each individual study was analyzed by calculating their transformed RR, SE, weight, as well as its total RR ratio and 95% CI. Additionally, total RR estimate and 95% CI (1.19 [1.10, 1.29]) were calculated. Heterogeneity as well as overall effect were assessed. To the right of the figure indicates the forest plot analysis. Larger effect sizes depicted larger red boxes. Smaller red boxes (Chen et al., 2005, Lee et al., 2016) corresponded to smaller effect sizes, however displayed wider 95% CI ranges with a longer horizontal line. Black diamond box described the total estimate of the analysis.

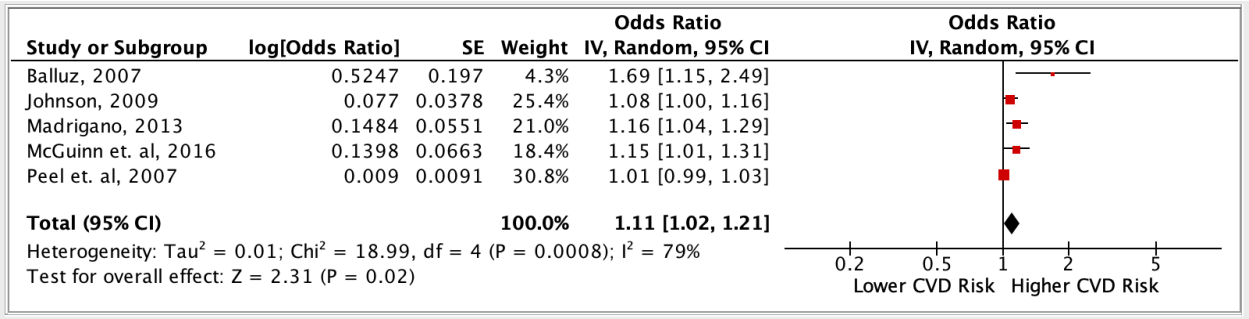

Figure 3. Odds Ratio summary estimate and forest plot. Figure 4 displays the summary estimate and forest plot analysis for the OR group. Each individual study was analyzed by calculating its transformed OR, SE, weight, as well as its total OR ratio and 95% CI. Additionally, total OR estimate and 95% CI (1.11 [1.02, 1.21]) were calculated. Heterogeneity as well as overall effect were assessed. Larger red boxes indicated larger effect sizes, while smaller red boxes (Balluz, 2007) indicated smaller effect sizes. Black diamond box described the total estimate of the analysis.

After testing summary effect estimates, a funnel plot analysis was performed to assess publication biases across all groups. The SMD funnel plot indicated asymmetry, as seen in figure 5. With the exception of two studies, most were clustered towards funnel's narrow peak, indicating larger effect sizes with small amounts of variance. Figure 6 depicts an accurate representation of a symmetrical funnel plot, as each plot has an even amount of studies on each quadrant. HR's funnel plot depicts one study on the bottom of the left quadrant, indicating greater variance and a more extensive spread around the summary effect size. The spread narrows with decreasing variance, indicating larger studies.

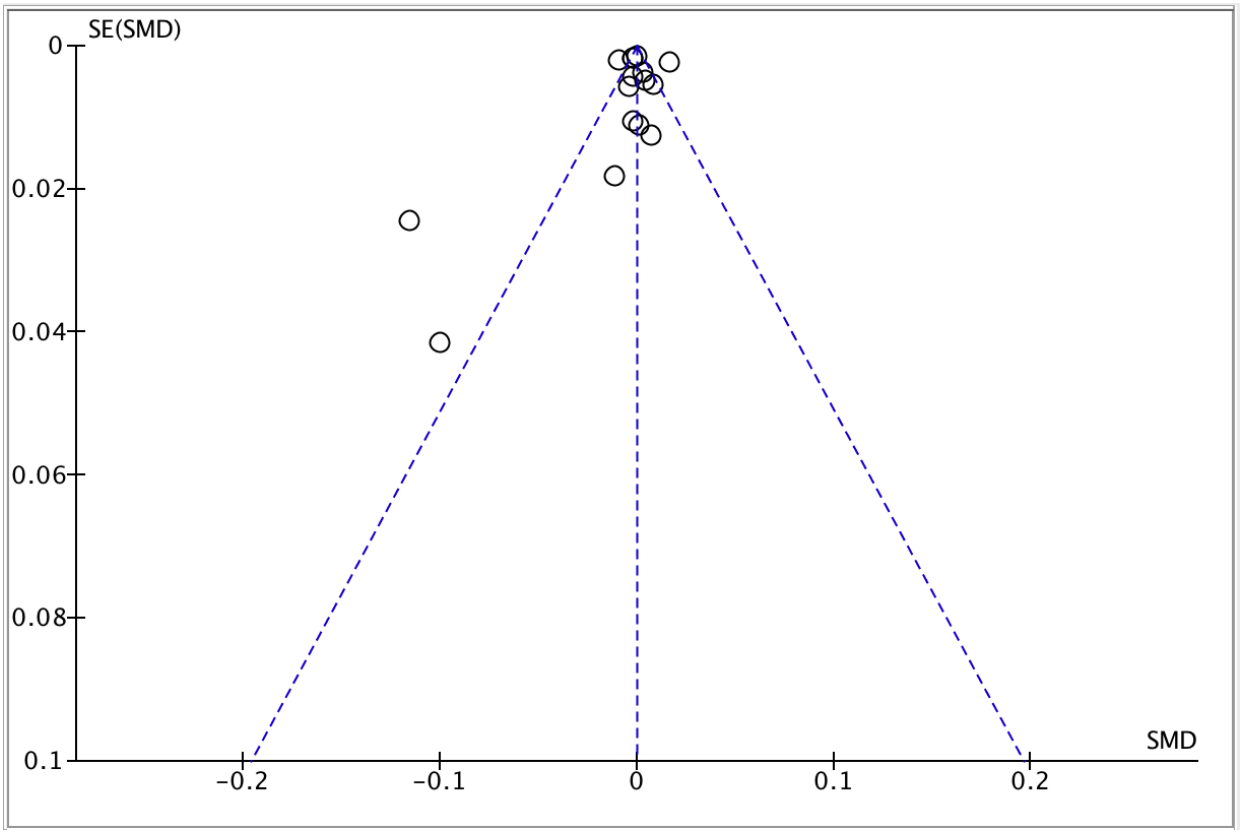

Figure 4. Standardized mean differences funnel plot. Figure 5 displays the SMD funnel plot analysis. RevMan 5.3 auto-generated a funnel plot analyzing all 16 studies as plot points (noted as clear circles). The plot shown indicates asymmetry, with missing plot points to the bottom quadrant of the triangle. Two plot points were outliers, located to the left of the plot. Plot points clumped at the apex of the funnel plot indicated larger effect sizes.

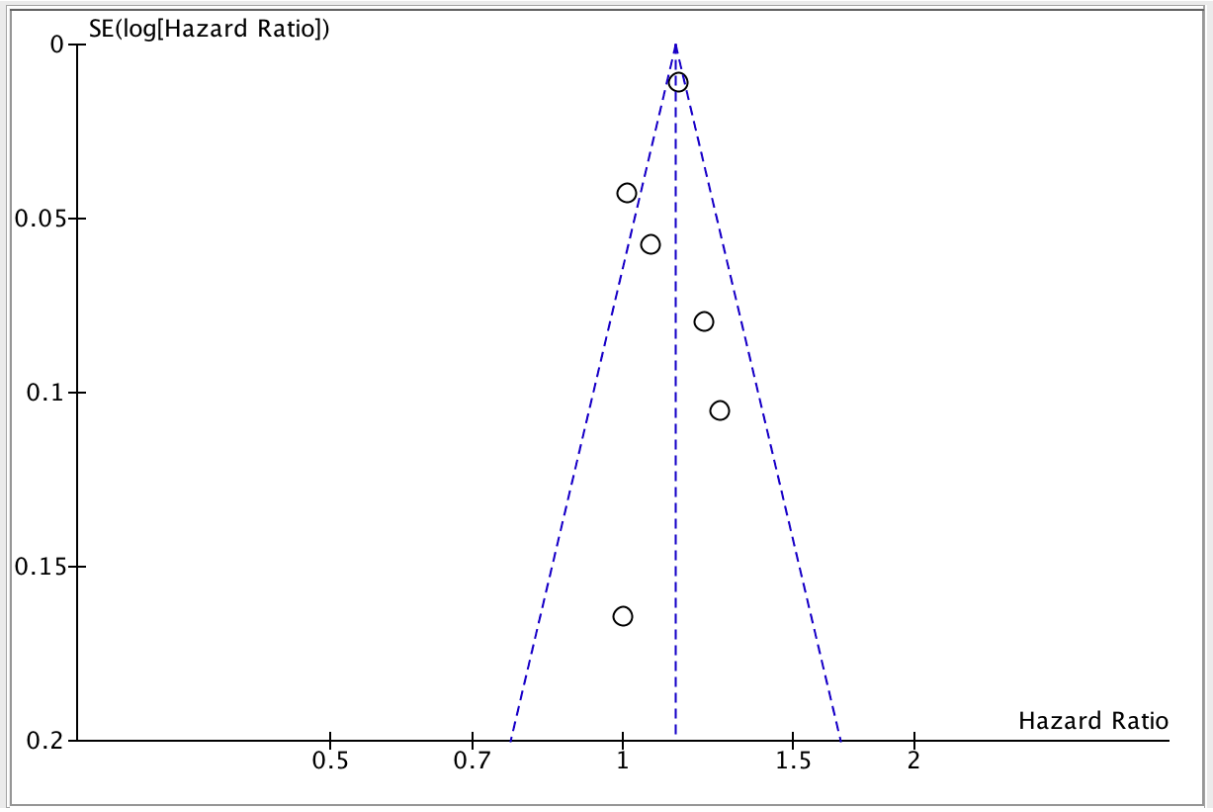

Figure 5. Hazard Ratio funnel plot. Figure 6 displays the HR group funnel plot analysis, which indicated symmetry. All quadrants were fulfilled with plot points, except for one outlier located to the left of the plot.

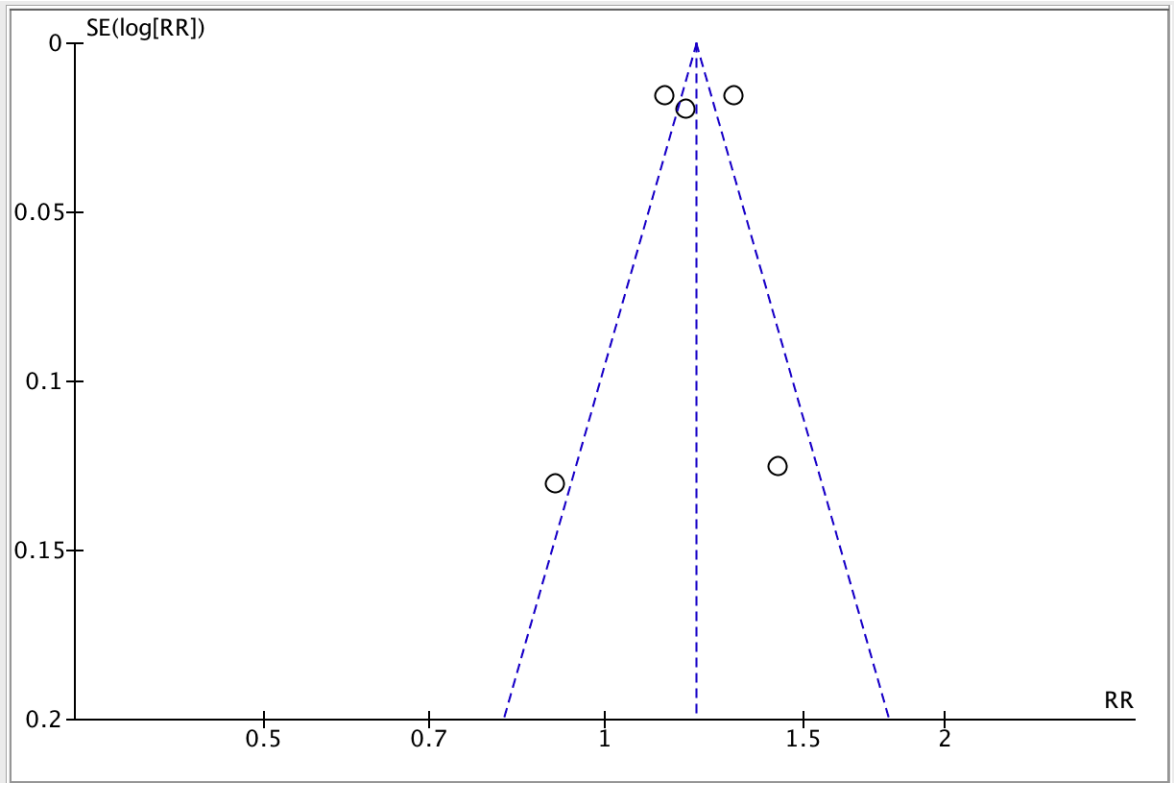

Figure 6. Risk Ratio funnel plot. Figure 7 displays the RR group funnel plot analysis, which indicated asymmetry. Asymmetry can be seen by missing plot points to the lower left quadrant. Three plot points were treated as outliers, located to the left and right of the plot.

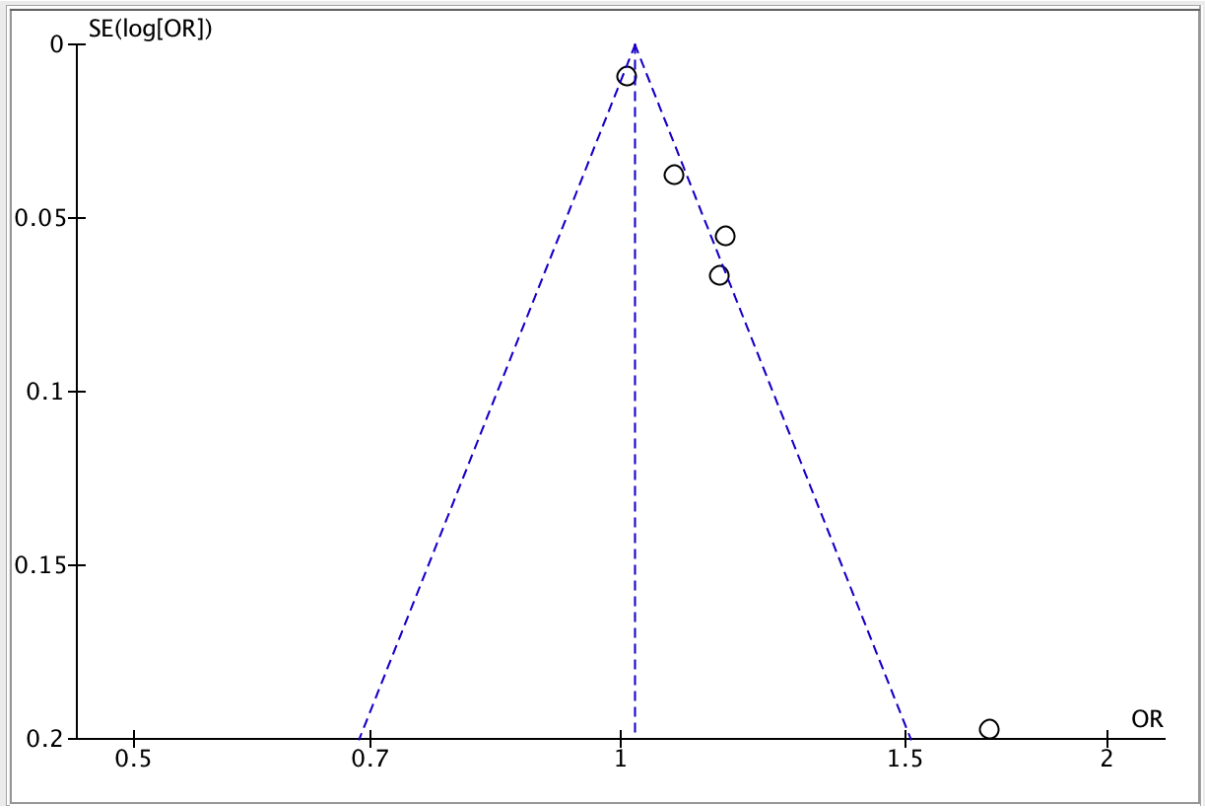

Figure 7. Odds Ratio funnel plot. Figure 8 displays the OR group funnel plot analysis, which indicated asymmetry. Plot points were missing to the left upper and lower quadrant, as most of the plot points were located to the upper right quadrant. There were two outliers located to the right of the plot.

Comparison boxplots were created to illustrate the difference between mean ratios and effect estimates for each study design. Ratios were averaged and plotted against each effect estimate. Alternatively, the OR group displayed the highest mean (1.218), which corresponded with the highest variance (0.255), while HR displayed the smallest mean (1.115) with the smallest variance (0.009). Additionally, the OR group displayed the most extensive range between minimum to maximum values. Formation of a second comparison boxplot aimed to minimize any potential skewed data through logarithmic transformation. Both plots remained similar in their descriptive statistic tables and their boxplots, suggesting consistency within the data.

Table 3: Descriptive statistics for Figure 9. Table 3 shows weighted ratios for each group. Weighted ratios can be found from the summary effect estimate section in figures 2-4. The bottom left of the table indicates the minimum, 1st interquartile range (Q1), median, 3rd interquartile range (Q3), maximum, mean, standard deviation, and variance values from each group, respectively.

	Hazard Ratio	***Risk Ratio***	***Odds Ratio***
	1.07	1.42	1.69
	1.21	1.13	1.08
	1.14	0.90	1.16
	1.26	1.18	1.15
	1.01	1.30	1.01
	1.00	-	-
Min	1.00	0.90	1.01
Q1	1.025	1.13	1.08
Median	1.105	1.18	1.15
Q3	1.1925	1.30	1.16
Max	1.26	1.42	1.69
Mean	1.115	1.186	1.218
SD	0.097	0.470	0.505
Variance	0.009	0.221	0.255

Table 4: Log-Transformed descriptive statistics for Figure 10. Table 4 shows weighted ratios for each group in its logarithmic transformed value. Transformed values can be found from the summary effect estimate section in figures 2-4. The bottom left of the table indicates the minimum, 1st interquartile range (Q1), median, 3rd interquartile range (Q3), maximum, mean, standard deviation, and variance values from each group, respectively.

	Hazard Ratio	***Risk Ratio***	***Odds Ratio***
	0.0677	0.3507	0.5247
	0.1906	0.1222	0.077
	0.131	-0.1	0.1484
	0.2311	0.1655	0.1398
	0.01	0.2624	0.009
	0.00	0.00	0.00
Min	0.00	-0.1	0.009
Q1	0.024425	0.1222	0.077
Median	0.09935	0.1655	0.1398
Q3	0.1757	0.2624	0.1484
Max	0.2311	0.3507	0.5247
Mean	0.1051	0.1602	0.1798
SD	0.08693	0.15226	0.17961
Variance	0.00756	0.02318	0.03226

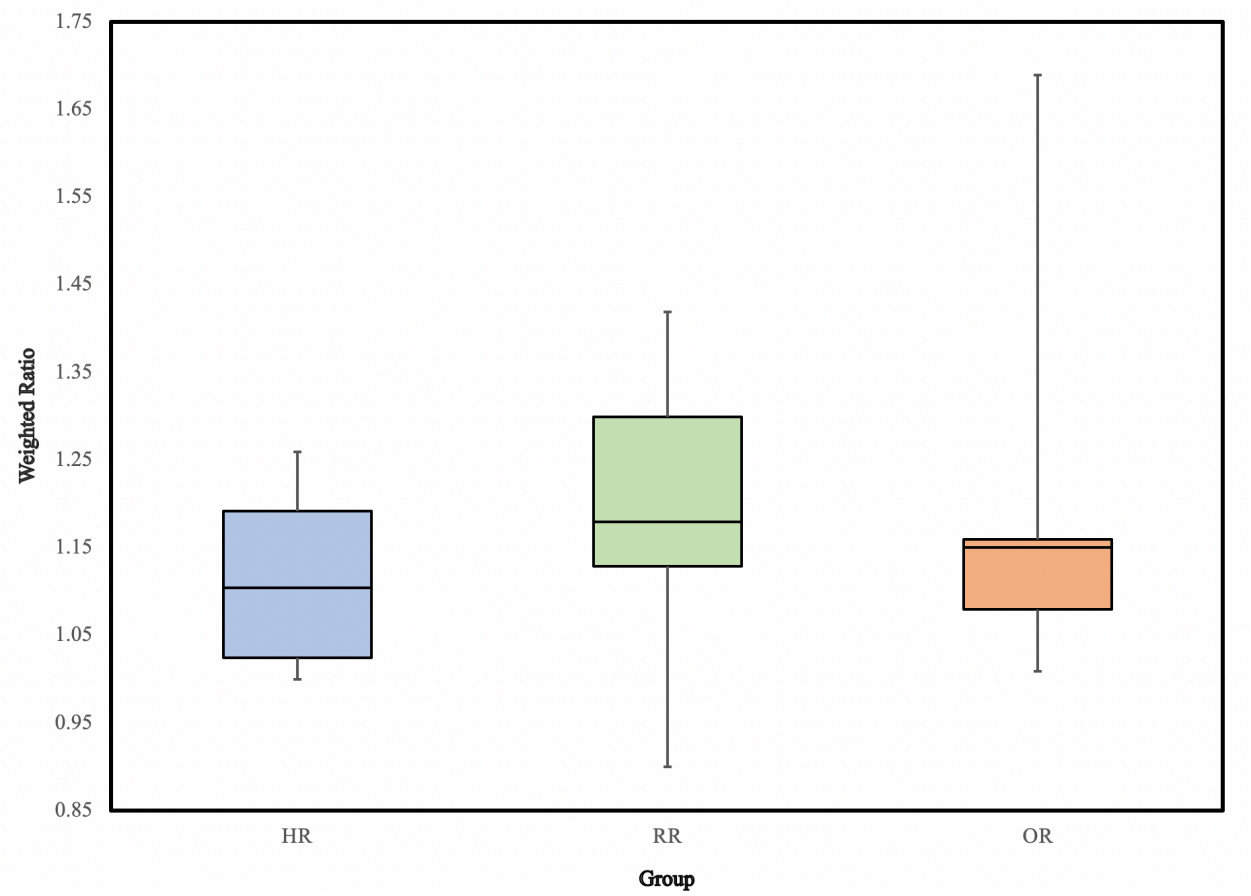

Figure 8. Weighted ratio box-and-whisker comparison plot. Figure 9 shows a weighted ratio box-and-whisker comparison plot of all groups. Horizontal lines across each box indicate the median values. Whiskers on each group indicate their maximum and minimum values respectively. Each group's weighted ratio values, minimum values, 1st interquartile range, median values, 3rd interquartile range can be found in Table 3.

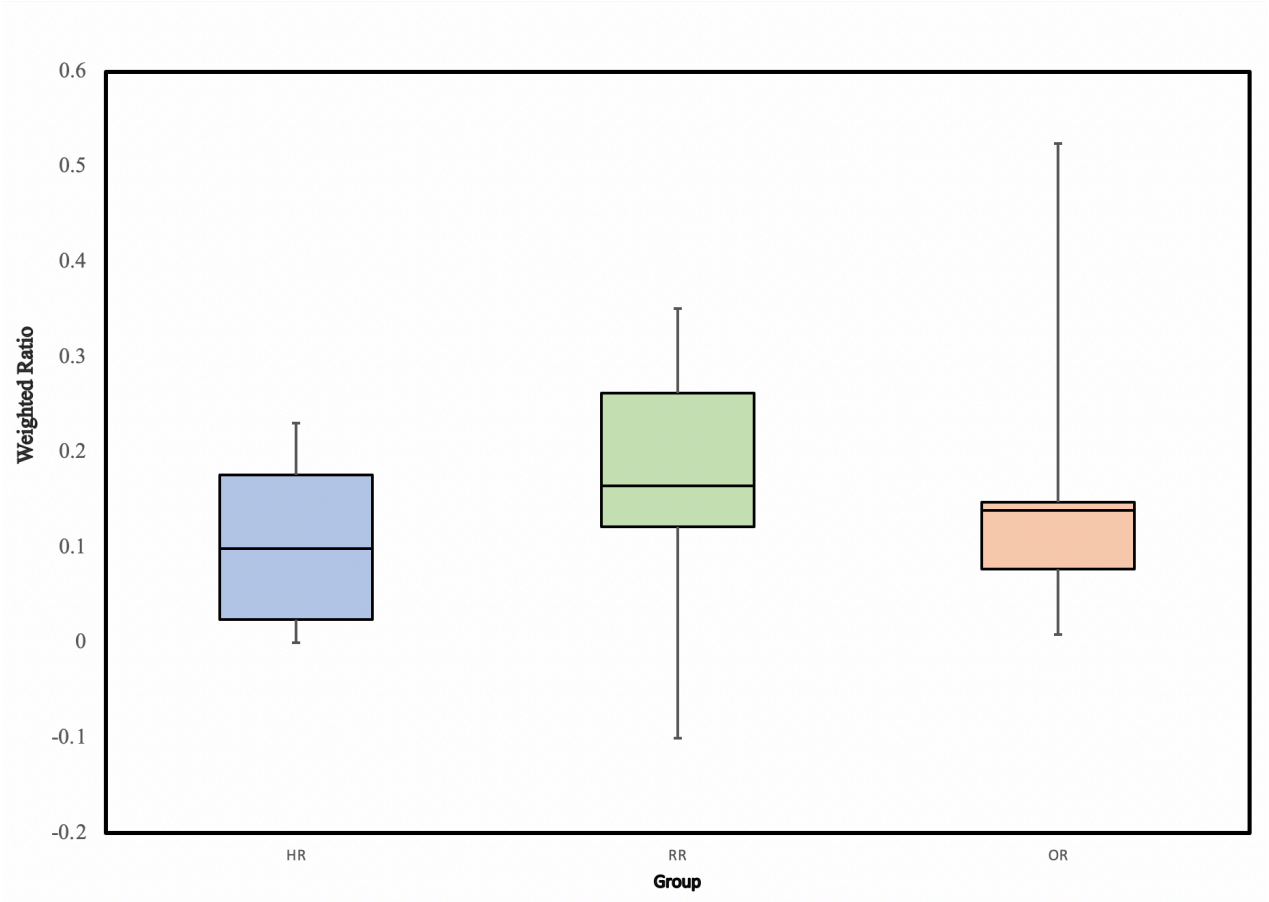

Figure 9. Weighted log-transformed box-and-whisker comparison plot. Figure 10 shows a weighted log-transformed box-and-whisker comparison plot of all groups. Horizontal lines across each box indicate the median values. Whiskers on each group indicate their maximum and minimum values respectively. Each group's weighted ratio values, minimum values, 1st interquartile range, median values, 3rd interquartile range can be found in Table 4. Weighted ratios were transformed on a $\log_{10}$ scale.

Chapter 5. Discussion and Conclusion

The purpose of this study aimed to investigate exposure of particulate matter and the risk of CVD byways of a meta-analysis. Hypothesized $PM_{2.5}$ exposure would increase CVD risk, as results from figures 2-4 indicated that $PM_{2.5}$ exposure positively associated risk of CVD development. A meta-analysis approach tested the hypothesis by combining multiple studies with different conclusions, ultimately forming one analysis with a novel conclusion. A random-effects model was introduced into this meta-analysis as the preferred method. Its conservative approach to the analysis compiled several studies into one analysis, providing a clear conclusion to the research question. A collection of 16 studies were analyzed as a whole to determine their effect size relative to each study by using SMD. In order to provide a more thorough analysis, each study was categorized based on its study design. Six studies involved time-series data, while ten studies used case-crossover studies. Time-series studies were primarily composed of HR, while half of the case-crossover studies involved RR, and the other half used OR.

Results from the data showed that exposure to particulate matter increases the risk of developing CVD. All groups provided further evidence through heterogeneity tests, forest plots, and funnel plots, as shown from figures 2-4. HR, RR and OR were significantly different ($p < 0.05$). While testing for summary effect estimates, all groups had greater than 54% on their I^2 heterogeneity tests, indicating moderate to significant heterogeneity (100,101). Further analysis showed small Tau^2 values in all 3 groups, with HR with the lowest (0.00), representing smaller variation between the true mean. Tau^2 in accordance with I^2 reflects the true heterogeneity in the study and indicates that the data are statistically different from each other. Chi^2 was also calculated, which is an additional testing for significance. Chi^2, or Q statistic, is used to estimate

the variance of true effects. Both HR and OR show similar Chi^2 values (10.80, 18.99), as there is clear excess in variation noted in RR (47.38). This can be translated to a smaller variance from the true mean.

After testing for heterogeneity, forest plots assessed the effect estimate of each group. The assessment was performed through heterogeneity tests and testing for the overall effect. HR mainly showed one study with a large effect size, while the rest of the studies were smaller in magnitude. In contrast, RR and OR mainly compromised of larger studies.

Publication bias is a significant threat to any meta-analysis as authors can show bias towards their study conclusions, which could negatively impact a meta-analysis. Publication bias can be noted in a funnel plot by its asymmetrical shape, through an uneven distribution of plots in each quadrant (101,102). Funnel plots were constructed in order to rule out any publication bias. In absence of bias and between-study heterogeneity, scattering will be due to sampling variation alone, and the plot will resemble a symmetrical inverted funnel. The HR group displayed a symmetrical funnel plot based on the funnel plot analysis results, with data points evenly distributed throughout the plot, as shown in figure 6. Smaller sized studies populated the plot's base while larger sized studies populated the top of the plot. In contrast, figures 7 and 8 displayed asymmetries in RR and OR. The RR group missed plots in the lower left quadrant, while the OR group missed plots in the left and right quadrants. Asymmetry was notably seen in the lower left quadrant of both funnel plots, indicating a missing smaller study. Although asymmetry normally corresponds to publication bias, there is more than one reason for

asymmetry, including using larger sample sizes. Larger effect sizes were noted at the narrow point of the plot in each effect estimate plot.

Some authors have argued that funnel plots' interpretation can be too subjective, with limited ability to correctly identify a true symmetric funnel plot. Differences in methodological quality are a potential source of funnel plot asymmetry. Smaller studies used by the original authors tend to be conducted and analyzed with less methodological rigor than larger studies. True heterogeneity in intervention effects could also lead to funnel plot asymmetry (103,104). The size of the effect differs according to the study size. A substantial benefit may be seen in patients with high exposure to particulate matter resulting in higher risk for CVD. These high-risk patients are more likely to be included in early, small studies (105–107). Additionally, groups that displayed higher statistical significance such as the OR group, could suggest that the cause of asymmetry are likely due to factors other than publication bias.

The creation of a summary box-and-whisker plot illustrated the three groups' effect against each other. Two plots were made, one being the average ratio of all groups, and a logarithmic-transformed graph of the ratios was used. The standard plot demonstrated a more extensive range in values in the OR group, while the HR group displayed much narrower values. Descriptive statistics on the plots indicated all three groups showed small variance values, with HR group displaying the smallest amount. Small variance values indicate that the data points tend to be close to the true mean. A logarithmic-transformed plot was used to remove any potential skews in the data, which indicated similar results from the first plot. Small differences

found in the first plot were more pronounced in the second plot, definitively conclude that all three groups are not identical but similar.

Significant limitations noticed from this project involved larger effect sized studies. Although larger effect sizes have advantages in reaching greater statistical significance, it can also distort the data, primarily seen from the funnel plots. While this study provided novel conclusions to exposure to $PM_{2.5}$ and CVD risk, further studies can aim to explore and identify any potential solutions to decrease $PM_{2.5}$ exposure in a given population in hopes of decreasing the risk of developing CVD. Particulate matter is a broad term that encompasses several types of ambient particles found in everyday life. Soot, for example, the common type of $PM_{2.5,}$ is mainly prevalent in urban cities such as Los Angeles. Further studies could investigate several alternative methods factories and fossil fuel burning industries can implement to decrease CVD's likelihood.

Bibliography

1 Heart Disease and Stroke Statistics – 2019 Update: A Report from the American Heart Association. 2019; 139(10): 57-528.

2 Heart Disease Facts. CDC. 2020. https://www.cdc.gov/heartdisease/facts.htm.

3 Mozaffarian D, Benjamin E, Go A, Arnett D, Blaha M, Cushman M, et al. Heart Disease and Stroke Statistics – 2016 Update. 2016; 133(4): 38-360.

4 CDC – DHDSP – Heart Disease – Oher Related Conditions – Cardiomyopathy. 2019. https://www.cdc.gov/heartdisease/cardiomyopathy.htm.

5 Dalen J, Albert J, Goldberg R, Weinstein R. The Epidemic of the 20th Century: Coronary Heart Disease. The American Journal of Medicine. 2014; 127(9): 807-812.

6 Sanz J, Fayad Z. Imaging of Atherosclerotic Cardiovascular Disease. Nature. 2008; 451: 953-957.

7 Finegold J, Asaria P, Francis D. Mortality from Ischemic Heart Disease by Country, Region, and Age: Statistics from World Health Organization and United Nations. International Journal of Cardiology. 2013; 168(2): 934-935.

8 Díez J, Frohlich E. A Translational Approach to Hypertensive Heart Disease. Hypertension. 2010; 55(1): 1-8.

9 Frohlich E, Apstein C, Chobanian A, Devereux R, Dustan H, Dzau V, et al. The Heart in Hypertension. New England Journal of Medicine. 1992; 327(14): 998-1008.

10 Kahan T, Bergfeldt L. Left Ventricular Hypertrophy in Hypertension: Its Arrhythmogenic Potential. Heart. 2005; 91(2): 250-256.

11 Maron B, McKenna W, Danielson G, Kappenberger L, Kuhn H, Seidman C, et al. American College of Cardiology/European Society of Cardiology Clinical Expert Consensus Document on Hypertrophic Cardiomyopathy: A Report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents and the European Society of Cardiology Committee for Practice Guidelines. Journal of the American College of Cardiology. 2003; 42(9): 1687-1713.

12 Kindermann I, Barth C, Mahfoud F, Ukena C, Lenski M, Yilmaz A, et al. Update on Myocarditis. Journal of the American College of Cardiology. 2012; 59(9): 779-792.

13 Little William C, Freeman Gregory L. Pericardial Disease. Circulation. 2006; 113(12): 1622-1632.

14 Enserro D, Vasan R., Xanthakis V. Twenty-year Trends in the American Heart Association Cardiovascular Health Score and Impact on Sub-clinical and Clinical Cardiovascular Disease: The Framingham Offspring Study. Journal of the American Heart Association. 2018. 7(11): e008741.

15 United States Surgeon General. The Health Consequences of Smoking – 50 Years of Progress: A Report of the Surgeon General. American Psychological Association; 2014. http://doi.apa.org/get-pe-doi.cfm?doi=10.1037/e510072014-001.

16 Kaminsky L, Arena R, Beckie T, Brubaker P, Church T, Forman D, et al. The Importance of Cardiorespiratory Fitness in the United States: The Need for a National Registry: A Policy Statement from the American Heart Association. Circulation. 2013; 127(5): 652-662.

17 Lloyd-Jones D, Hong Y, Labarthe D, Mozaffarian D, Appel L, Van Horn L, et al. Defining and Setting National Goals for Cardiovascular Health Promotion and Disease Reduction. Circulation. 2010; 121(4): 586-613.

18 Poirier P, Giles T, Bray G, Hong Y, Stern J, Pi-Sunyer F, et al. Obesity and Cardiovascular Disease: Pathophysiology, Evaluation, and Effect of Weight Loss. Circulation. 2006; 113(6): 898-918.

19 Ford E, Capewell S. Trends in Total and Low-density Lipoprotein Cholesterol among U.S. Adults: Contributions of Changes in Dietary Fat Intake and Use of Cholesterol-lowering Medications. Plos One. 2013; 8(5): e65228.

20 Crim M, Yoon S, Ortiz E, Wall H, Schober S, Gillespie C, et al. National Surveillance Definitions for Hypertension Prevalence and Control among Adults. Circulation: Cardiovascular Quality and Outcomes. 2012; 5(3): 343-351.

21 Fox C, Golden S, Anderson C, Bray G, Burke L, De Boer I, et al. Update on Prevention of Cardiovascular Disease in Adults with Type 2 Diabetes Mellitus in Light of Recent Evidence: A Scientific Statement from the American Heart Association and the American Diabetes Association. Diabetes Care. 2015; 38(9): 1777-1803.

22 Forounzanfar M, Afshin A, Alexander L, Anderson H, Bhutta Z, Biryukov S, et al. Global, Regional, and National Comparative Risk Assessment of 79 Behavioral, Environmental, Occupational, and Metabolic Risks or Clusters of Risks, 1990-2015: A Systemic Analysis for the Global Burden of Disease Study 2015. The Lancet. 2016; 388(10053): 1659-1724.

23 Go A, Mozaffarian D, Roger V, Benjamin E, Berry J, Borden W, et al. Executive Summary: Heart Disease and Stroke Statistics – 2013 Update. Circulation. 2013; 127(1): 143-152.

24 Bureau UC. Population Trends in Incorporated Places: 2000 to 2013. The United States Census Bureau. 2020. https://www.census.gov/library/publications/2015/demo/p25-1142.html.

25 Bureau UC. U.S. Cities Home to 62.7% of Population but Comprise 3.5% of Land Area. The United States Census Bureau. 2019. https://www.census.gov/newsroom/press-releases/2015/cb15-33.html.

26 U.S. Cities Factsheet. Center for Sustainable Systems. 2019. https://css.umich.edu/factsheets/us-cities-factsheet.

27 Sweta B. Risk Factors of Cardiovascular Disease between Urban and Rural Adult Population. International Journal of Caring Sciences. 2018; 11(1): 71-86.

28 Kulshreshtha A, Goyal A, Dabhadkar K, Veledar E, Vaccarino V. Urban-rural Differences in Coronary Heart Disease Mortality in the United States: 1999-2009. Public Health Rep. 2014; 129(1): 19-29.

29 Pope C, Thun M, Namboodiri M, Dockery D, Evans J, Speizer F, et al. Particulate Air Pollution as a Predictor of Mortality in a Prospective Study of U.S. Adults. American Journal of Respiratory and Critical Care Medicine. 1995; 151(3): 669-674.

30 III CAP, Dockery D. Health Effects of Fine Particulate Air Pollution: Lines that Connect. Journal of the Air & Waste Management Association. 2006; 56(6): 706-742.

31 Brook R, Rajagopalan S, Pope C, Brook J, Bhatnagar A, Diez-Roux A, et al. Particulate Matter Air Pollution and Cardiovascular Disease: An Update to the Scientific Statement from the American Heart Association. Circulation. 2010; 121(21): 2331-2378.

32 Brook R, Franklin B, Cascio W, Hong Y, Howard G, Lipsett M, et al. Air Pollution and Cardiovascular Disease: A Statement for Healthcare Professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. Circulation. 2004; 109(21): 2655-2671.

33 McGuinn L, Ward-Caviness C, Neas L, Schneider A, Di Q, Chudnovsky A, et al. Fine Particulate Matter and Cardiovascular Disease: Comparison of Assessment Methods for Long-term Exposure. Environmental Research. 2017; 159: 16-23

34 Peters A, Dockery D, Muller J, Mittleman M. Increased Particulate Air Pollution and the Triggering of Myocardial Infarction. Circulation. 2001; 103(23): 2810-2815.

35 Simkhovich B, Kleinman M, Kloner R. Air Pollution and Cardiovascular Injury Epidemiology, Toxicology, and Mechanisms. Journal of the American College of Cardiology. 2008; 52(9): 719-726.

36 Münzel T, Gori T, Al-Kindi S, Deanfield J, Lelieveld J, Daiber A, et al. Effects of Gaseous and Solid Constituents of Air Pollution on Endothelial Function. European Heart Journal. 2018; 39(38): 3543-3550.

37 Ikeda M, Watari K, Suzuki M, Ito T, Yamasaki H, Sagai M, et al. Mechanism of Pathophysiological Effects of Diesel Exhaust Particles on Endothelial Cells. Environmental Toxicology and Pharmacology. 1998; 6(2): 117-123.

38 Finch J, Conklin D. Air Pollution-induced Vascular Dysfunction: Potential Role of Endothelin-1 (ET-1) System. Cardiovascular Toxicology. 2016; 16(3): 260-275.

39 Du Y, Xu X, Chu M, Guo Y, Wang J. Air Particulate Matter and Cardiovascular Disease: The Epidemiological, Biomedical and Clinical Evidence. Journal of Thoracic Disease. 2016; 8(1): 8-19.

40 Rückerl R, Ibald-Mulli A, Koenig W, Schneider A, Woelke G, Cyrys J, et al. Air Pollution and Markers of Inflammation and Coagulation in Patients with Coronary Heart Disease. American Journal of Respiratory and Critical Care Medicine. 2006; 173(4): 432-441.

41 Suwa T, Hogg J, Quinlan K, Ohgami A, Vincent R, Van Eeden S. Particulate Air Pollution Induces Progression of Atherosclerosis. Journal of the American College of Cardiology. 2002; 39(6): 935-942.

42 Sørensen M, Daneshvar B, Hensen M, Dragsted L, Hertel O, Knudsen L, et al. Personal $PM_{2.5}$ Exposure and Markers of Oxidative Stress in Blood. Environmental Health Perspect. 2003; 111(2): 161-166.

43 Przyklenk K, Kloner R. "Reperfusion Injury" by Oxygen-Derived Free Radicals? Effect of Superoxide Dismutase Plus Catalase, given at the Time of Reperfusion, on Myocardial Infarct Size, Contractile Function, Coronary Microvasculature, and Regional Myocardial Blood Flow. Circulation Research. 1989; 64(1): 86-96.

44 Langrish J, Bosson J, Unosson J, Muala A, Newby D, Mills N, et al. Cardiovascular Effects of Particulate Air Pollution Exposure: Time Course and Underlying Mechanisms. Journal of Internal Medicine. 2012; 272(3): 224-239.

45 Nelin T, Joseph A, Gorr M, Wold L. Direct and Indirect Effects of PM on the Cardiovascular System. Toxicology Letters. 2012; 208(3): 293-299.

46 Tibuakuu M, Jones M, Navas-Acien A, Zhao D, Guallar E, Gassett A, et al. Exposure to Ambient Air Pollution and Calcification of the Mitral Annulus and Aortic Valve: The Multi-Ethnic Study of Atherosclerosis (MESA). Environmental Health. 2017; 16(133).

47 Donaldson K, Stone V, Seaton A, MacNee W. Ambient Particle Inhalation and the Cardiovascular System: Potential Mechanisms. Environmental Health Perspect. 2001; 109(4): 523-527.

48 Bolli R, Zughaib M, Li X, Tang X, Sun J, Triana J, et al. Recurrent Ischemia in the Canine Heart Causes Recurrent Bursts of Free Radical Production that have a Cumulative Effect on

Contractile Function. A Pathophysiological Basis for Chronic Myocardial “Stunning”. Journal of Clinical Investigation. 1995; 96(2): 1066-1084.

49 Volk T, Schmutzler M, Engelhardt L, Pantke U, Laule M, Stangl K, et al. Effects of Different Steroid Treatment of Reperfusion-associated Production of Reactive Oxygen Species and Arrhythmias during Coronary Surgery. Acta Anaesthesiologica Scandinavica. 2003; 47(6): 667-674.

50 III CAP, Bhatnagar A, McCracken J, Abplanalp W, Conklin D, O’Toole T. Exposure to Fine Particulate Air Pollution is Associated with Endothelial Injury and Systemic Inflammation. Circulation Research. 2016; 119: 1204-1214.

51 Van Eeden S, Yeung A, Quinlam K, Hogg J. Systemic Response to Ambient Particulate Matter. Proceedings of the American Thoracic Society. 2005; 2(1): 61-67.

52 Kaufman J, Adar S, Barr R, Budoff M, Burke G, Curl C, et al. Association between Air Pollution and Coronary Artery Calcification within Six Metropolitan Areas in the USA (The Multi-ethnic Study of Atherosclerosis and Air Pollution): A Longitudinal Cohort Study. Lancet. 2016; 388(10045): 696-704.

53 Brook R. Cardiovascular Effects of Air Pollution. Clinical Science. 2008; 115(6): 175-187.

54 Li Y, Rittenhouse-Olson K, Scheider W, Mu L. Effect of Particulate Matter Air Pollution on C-reactive Protein: A Review of Epidemiologic Studies. Reviews of Environmental Health. 2012; 27(2-3): 133-149.

55 Green R, Broadwin R, Malig B, Basu R, Gold E, Qi L, et al. Long- and Short-term Exposure to Air Pollution and Inflammatory/Hemostatic Markers in Midlife Women. Epidemiology. 2016; 27(2): 211-220.

56 Li W, Dorans K, Wilker E, Rice M, Ljungman P, Schwartz J, et al. Short-term Exposure to Ambient Air Pollution and Biomarkers of Systemic Inflammation. Arteriosclerosis, Thrombosis, and Vascular Biology. 2017; 37(9): 1793-1800.

57 Jaafari J, Naddafi K, Yunesian M, Nabizadeh R, Hassanvand M, Shamsipour M, et al. The Acute Effects of Short-term Exposure to Particulate Matter from Natural and Anthropogenic Sources on Inflammation and Coagulation Markers in Healthy Young Adults. Science of The Total Environment. 2020; 735: 139427.

58 Fang S, Eisen E, Cavallari J, Mittleman M, Christiani D. Circulating Adhesion Molecules after Short-term Exposure to Particulate Matter among Welders. Occupational and Environmental Medicine. 2010; 67(1): 11-16.

59 Alexeeff S, Coull B, Gryparis A, Suh H, Sparrow D, Vokonas P, et al. Medium-term Exposure to Traffic-related Air Pollution and Markers of Inflammation and Endothelial Function. Environmental Health Perspect. 2011; 119(4): 481-486.

60 Pope C, Bhatnagar A, McCracken J, Abplanalp W, Conklin D, O'Toole T. Exposure to Fine Particulate Air Pollution is Associated with Endothelial Injury and Systemic Inflammation. Circulation Research. 2016; 119(11): 1204-1214.

61 McMurray J, Chopa M, Abdullah I, Smith W, Dargie H. Evidence of Oxidative Stress in Chronic Heart Failure in Humans. European Heart Journal. 1993; 14(11): 1493-1498.

62 Provost E, Madhloum N, Int P, De Boever P, Nawrot T. Carotid Intima-media Thickness, a Marker of Sub-clinical Atherosclerosis, and Particulate Air Pollution Exposure: The Meta-analytical Evidence. Plos One. 2015; 10(5): e0127014.

63 Zhang Z, Chan T, Guo C, Chang L, Lin C, Chuang Y, et al. Long-term Exposure to Ambient Particulate Matter ($PM_{2.5}$) is Associated with Platelet Counts in Adults. Environmental Pollution. 2018; 240: 432-439.

64 Gondalia R, Holliday K, Baldassari A, Justice A, Stewart J, Liao D, et al. Leukocyte Traits and Exposure to Ambient Particulate Matter Air Pollution in the Women's Health Initiative and Atherosclerosis Risk in Communities Study. Environmental Health Perspect. 2020; 128(1).

65 Valavanidis A, Fiotakis K, Vlachogianni T. Airborne Particulate Matter and Human Health: Toxicological Assessment and Importance of Size and Composition of Particles for Oxidative Damage and Carcinogenic Mechanisms. Journal of Environmental Science and Health, Part C Environmental Carcinogenesis Ecotoxicology Reviews. 2008; 26(4): 339-362.

66 Shakya K, Peltier R, Zhang Y, Pandey B. Roadside Exposure and Inflammation Biomarkers among a Cohort of Traffic Police in Kathmandu, Nepal. International Journal of Environmental Research and Public Health. 2019; 16(3): 377.

67 Tsai D, Amyai N, Marques V, Wang J, Riediker M, Mooser V, et al. Effects of Particulate Matter on Inflammatory Markers in the General Adult Population. Part Fibre Toxicology. 2012; 9:24.

68 Mozos I, Malainer C, Horbańczuk J, Gug C, Stoian D, Luca C, et al. Inflammatory Markers for Arterial Stiffness in Cardiovascular Diseases. Frontiers in Immunology. 2017; 8: 1058.

69 Sawyer D. Oxidative Stress in Heart Failure: What are we missing? American Journal of the Medical Sciences. 2011; 342(2): 120-124.

70 Geiser M, Rothen R, Kapp N, Schürch S, Kreyling W, Schulz H, et al. Ultrafine Particles Cross Cellular Membranes by Non-phagocytic Mechanisms in Lungs and in Cultured Cells. Environmental Health Perspect. 2005; 113(11): 1555-1560.

71 Nemmar A, Hoet P, Vanquickenborne B, Dinsdale D, Thomeer M, Hoylaerts M, et al. Passage of Inhaled Particles into the Blood Circulation in Humans. Circulation. 2002; 105(4): 411-414.

72 Shimada A, Kawamura N, Okajima M, Kaewamatawong T, Inoue H, Morita T. Translocation Pathway of the Intratracheally Instilled Ultrafine Particles from the Lung into the Blood Circulation in the Mouse. Toxicologic Pathology. 2006; 34(7): 949-957.

73 Brown J, Zeman K, Bennett W. Ultrafine Particle Deposition and Clearance in the Healthy and Obstructed Lung. American Journal of Respiratory and Critical Care Medicine. 2002; 166(9): 1240-1247.

74 Chen R, Qiao L, Li H, Zhao Y, Zhang Y, Xu W, et al. Fine Particulate Matter Constituents, Nitric Oxide Synthase DNA Methylation and Exhaled Nitric Oxide. Environmental Science and Technology. 2015; 49(19): 11859-11865.

75 Zanobetti A, Gold D, Stone P, Suh H, Schwartz J, Coull B, et al. Reduction in Heart Rate Variability with Traffic and Air Pollution in Patients with Coronary Artery Disease. Environmental Health Perspect. 2010; 118(3): 324-330.

76 Bhatnagar A. Environmental Cardiology: Studying Mechanistic Links between Pollution and Heart Disease. Circulation Research. 2006; 99(7): 692-705.

77 Stein P, Barzilay J, Chaves P, Mistretta S, Domitrovich P, Gottdiener J, et al. Novel Measures of Heart Rate Variability Predict Cardiovascular Mortality in Older Adults Independent of Traditional Cardiovascular Risk Factors. Journal of Cardiovascular Electrophysiology. 2008; 19(11): 1169-1174.

78 Sessa F, Anna V, Messina G, Cibelli G, Monda V, Marsala G, et al. Heart Rate Variability as Predictive Factor for Sudden Cardiac Death. Aging. 2018; 10(2): 166-167.

79 Yeatts K, Svendsen E, Creason J, Alexis N, Herbst M, Scott J, et al. Coarse Particulate Matter ($PM_{2.5\text{-}10}$) Affects Heart Rate Variability, Blood Lipids, and Circulating Eosinophils in Adults with Asthma. Environmental Health Perspect. 2007; 115(5): 709-714.

80 Bartell S, Longhurst J, Tjoa T, Sioutas C, Delfino R. Particulate Air Pollution, Ambulatory Heart Rate Variability, and Cardiac Arrhythmia in Retirement Community Residents with Coronary Artery Disease. Environmental Health Perspect. 2013; 121(10): 1135-1141.

81 Pope C, Turner M, Burnett R, Jerrett M, Gapstur S, Diver W, et al. Relationships between Fine Particulate Air Pollution, Cardiometabolic Disorders, and Cardiovascular Mortality. Circulation Research. 2015; 116(1): 108-115.

82 Puett R, Hart J, Suh H, Mittleman M, Laden F. Particulate Matter Exposures, Mortality, and Cardiovascular Disease in the Health Professionals Follow-up Study. Environmental Health Perspect. 2011; 119(8): 1130-1135.

83 Wellenius G, Schwartz J, Mittleman M. Particulate Air Pollution and Hospital Admissions for Congestive Heart Failure in Seven United States Cities. American Journal of Cardiology. 2006; 97(3): 404-408.

84 Pope C, Muhlestein J, May H, Renlund D, Anderson J, Horne B. Ischemic Heart Disease Events Triggered by Short-term Exposure to Fine Particulate Air Pollution. Circulation. 2006; 114(23): 2443-2448.

85 Lipsett M, Ostro B, Reynolds P, Goldberg D, Hertz A, Jerrett M, et al. Long-term Exposure to Air Pollution and Cardiorespiratory Disease in the California Teachers Study Cohort. American Journal of Respiratory and Critical Care Medicine. 2011; 184(7): 828-835.

86 Miller K, Siscovick D, Sheppard L, Shepherd K, Sullivan J, Anderson G, et al. Long-term Exposure to Air Pollution and Incidence of Cardiovascular Events in Women. New England Journal of Medicine. 2007; 356(5): 447-458.

87 Puett R, Hart J, Yanosky J, Paciorek C, Schwartz J, Suh H, et al. Chronic Fine and Coarse Particulate Exposure, Mortality, and Coronary Heart Disease in the Nurses' Health Study. Environmental Health Perspect. 2009; 117(11): 1697-1701.

88 Weichenthal S, Villeneuve P, Burnett R, Van Donkelaar A, Martin R, Jones R, et al. Long-term Exposure to Fine Particulate Matter: Association with Non-accidental and Cardiovascular Mortality in the Agricultural Health Study Cohort. Environmental Health Perspect. 2014; 122(6): 609-615.

89 Chen L, Knutsen S, Shavlik D, Beeson W, Petersen F, Ghamsary M, et al. The Association between Fatal Coronary Heart Disease and Ambient Particulate Air Pollution: Are Females at Greater Risk? Environmental Health Perspect. 2005; 113(12): 1723-1729.

90 Gan W, Koehoorn M, Davies H, Demers P, Tamburic L, Brauer M. Long-term Exposure to Traffic-related Air Pollution and the Risk of Coronary Artery Disease Hospitalization and Mortality. Environmental Health Perspect. 2011; 119(4): 501-507.

91 Lee M, Koutrakis P, Coull B, Kloog I, Schwartz J. Acute Effect of Fine Particulate Matter on Mortality in Three Southeastern States 2007-2011. Journal of Exposure Science and Environmental Epidemiology. 2016; 26(2): 173-179.

92 Pope C, Burnett R, Thurston G, Thun M, Calle E, Krewski D, et al. Cardiovascular Mortality and Long-term Exposure to Particulate Air Pollution. Circulation. 2004; 109(1): 71-77.

93 Balluz L, Wen X, Town M, Shire J, Qualter J, Mokdad A. Ischemic Heart Disease and Ambient Air Pollution of $PM_{2.5}$ in 51 Counties in the U.S. Public Health Reviews. 2007; 122(5): 626-633.

94 Johnson D, Parker J. Air Pollution Exposure and Self-reported Cardiovascular Disease. Environmental Research. 2009; 109(5): 582-589.

95 Madrigano J, Kloog I, Goldberg R, Coull B, Mittleman M, Schwartz J. Long-term Exposure to $PM_{2.5}$ and Incidence of Acute Myocardial Infarction. Environmental Health Perspect. 2013; 121(2): 192-196.

96 McGuinn L, Ward-Caviness C, Neas L, Schenider A, Diaz-Sanchez D, Cascio W, et al. Association between Satellite-based Estimates of Long-term $PM_{2.5}$ Exposure and Coronary Artery Disease. Environmental Research. 2016; 145: 9-17.

97 Peel J, Metzger K, Klein M, Flanders W, Mulholland J, Tolbert P. Ambient Air Pollution and Cardiovascular Emergency Department Visits in Potentially Sensitive Groups. American Journal of Epidemiology. 2007; 165(6): 625-633.

98 Higgins J, Green S. The Generic Inverse Variance Outcome Type in RevMan. Cochrane Handbook. 2011. https://handbook-5-1.cochrane.org/chapter_9/9_4_3_2_the_generic_inverse_variance_outcome_type_in_revman.htm

99 Higgin J, Green S. Standard Errors from Confidence Intervals and P-values: Difference Measures. Cochrane Handbook. 2011. https://handbook-5-1.cochrane.org/chapter_7/7_7_7_2_obtaining_standard_errors_from_confidence_intervals_and.htm

100 Higgins J, Thompson S. Quantifying Heterogeneity in a Meta-analysis. Statistics in Medicine. 2002; 21(11): 1539-1558.

101 Higgins J, Green S. Identifying and Measuring Heterogeneity. Cochrane Handbook. 2011. https://handbook-5-1.cochrane.org/chapter_9/9_5_2_identifying_and_measuring_heterogeneity.htm

102 Terrin N, Schmid C, Lau J, Olkin I. Adjusting for Publication Bias in the Presence of Heterogeneity. Statistics in Medicine. 2003; 22(13): 2113-2116.

103 Sterne J, Egger M. Funnel Plots for Detecting Bias in Meta-analysis: Guidelines on Choice of Axis. Journal of Clinical Epidemiology. 2001; 54(10): 1046-1055.

104 Tang J, Liu J. Misleading Funnel Plot for Detection of Bias in Meta-analysis. Journal of Clinical Epidemiology. 2000; 53(5): 477-484.

105 Villar J, Piaggio G, Carroli G, Donner A. Factors Affecting the Comparability of Meta-analyses and Largest Trials Results in Perinatology. Journal of Clinical Epidemiology. 1997; 50(9): 997-1002.

106 Rücker G, Schwarzer G, Carpenter J, Binder H, Schumacher M. Treatment-effect Estimates Adjusted for Small-study Effects via a Limit Meta-analysis. Biostatistics. 2011; 12(1): 122-142.

107 Glasziou P, Irwig L. An Evidence-based Approach to Individualizing Treatment. British Medical Journal. 1995; 311(7016): 1356-1359.