Association Between Long-Term $PM_{2.5}$ Exposure and Subclinical Atherosclerosis in Middle-Aged Women

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Abstract

Air pollution (AP) is associated with an increased risk of cardiovascular disease (CVD). Subclinical atherosclerosis (SCA), a pre-clinical stage of CVD, is measured using coronary artery calcification (CAC). Agatston score correlates to the degree of SCA. Particulate matter ($\leq$2.5μm[$PM_{2.5}$]), a major component of AP, is known to affect CVD. We focused on two unique groups to study the association between $PM_{2.5}$ and CAC, including a group of women with polycystic ovary syndrome (PCOS), and women undergoing the menopausal transition (MT).

The first paper is a review of long-term $PM_{2.5}$ and SCA. The focus was on the epidemiological designs, populations, exposure measurement methodologies and results of the studies of $PM_{2.5}$ and SCA with an emphasis on women. Two studies that employed spatiotemporal exposure assessment provided evidence of an effect of $PM_{2.5}$ on CAC, whereas the remaining five did not provide such evidence. Postmenopausal women showed stronger associations between $PM_{2.5}$ and CAC. Overall, these investigations did not address the association of $PM_{2.5}$ and CAC risk in a systematic and rigorous way.

The second paper involved a case-control study of women with PCOS and their controls, a Pittsburgh based study (N=301). There was an association between long-term $PM_{2.5}$ and CAC in women with PCOS (OR=1.44; 95%CI: 1.02-2.05), adjusting for age and BMI, but this was not

evident among controls. Interaction test of PCOS and $PM_{2.5}$ on CAC suggest the positive association is stronger among PCOS women.

The last paper studied CAC and $PM_{2.5}$ in women undergoing MT using both a cross-sectional and a longitudinal design within the Study of Women Health Across the Nation (SWAN) Heart (N=366). There was no association between $PM_{2.5}$ and CAC. With a 2.2-year follow-up, MT (measured by menopausal status and time to final menstrual period) did not have significant effect on the association of $PM_{2.5}$ and CAC presence or progression in middle-aged women.

These findings suggest the need for further in-depth study of the association between long-term $PM_{2.5}$ and CAC during MT and of metabolically vulnerable populations like women with PCOS, as the public health impact of $PM_{2.5}$ to these populations might contribute to the burden of disease.

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Preface

I would like to thank my family, friends, and mentors for their help, support, and encouragement during my doctoral training in Epidemiology at the University of Pittsburgh.

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1.0 Introduction

Air pollution in the U.S. was responsible for an estimated 15,612 deaths in females, 1999-2015 (1). The harmful impact of air pollution relates to an increased risk in cardiovascular disease (CVD), which has been the leading killer for decades of all U.S. people and the nation's costliest chronic disease with an estimated cost of $555 billion in 2016 (1). Inhalational exposure to particulate matter with an aero diameter <2.5 μm ($PM_{2.5}$) triggers inflammation and generates reactive oxygen species release, leading to endothelial dysfunction, and has a detrimental effect on vascular cells (2–5). Coronary artery calcification (CAC) is a significant marker of subclinical atherosclerosis (SCA) and coronary heart disease (CHD) and has been found to be strongly correlated with the severity of atherosclerosis (6,7). Observational studies reported inconsistent findings on the relationship between long-term $PM_{2.5}$ exposure and CAC (8–14). In summary, those studies conducted earlier reported no associations (8,9,11–13), and those more recently published found that $PM_{2.5}$ is associated with an increased risk of CAC presence and progression among the overall population and $PM_{2.5}$ adds to the effect of CAC on several susceptible subgroups, including women and postmenopausal women (10,14). The findings are consistent with previous observational studies reporting that CHD risk increases after menopause (15,16). However, no study has evaluated the effect of $PM_{2.5}$ on CAC in middle-aged women. This is an important gap since previous studies suggest women are particularly vulnerable to atherosclerosis risk as they transition through menopause. Thus, the impact of $PM_{2.5}$ on atherosclerosis could be heightened during this vulnerable period. By investigating the association between long-term $PM_{2.5}$ exposure and CAC among middle-aged women, we can determine if menopause contributes to the stronger association between $PM_{2.5}$ and CAC observed in postmenopausal women, or if that

stronger association is due to chronological aging. Observational studies focusing on middle-aged women could critically advance understanding of the impact of air pollution on women's cardiovascular health. In addition, women with polycystic ovary syndrome (PCOS), a heterogeneous disorder involving altered ovarian function and affecting both reproductive and metabolic abnormalities that results in an increased risk of CHD, are another population who are at increased risk of elevated levels of metabolic symptoms and CAC (17–19). Hence, women with PCOS could be more sensitive to the $PM_{2.5}$ exposure effect on CAC risk than women without. The purpose of this work is to review the literature related to the association of long-term $PM_{2.5}$ exposure and CAC in middle-aged women to identify research gaps. We then evaluated the effect of long-term $PM_{2.5}$ on CAC using two unique study populations, one from the Study of Women's Health across the Nation (SWAN) Heart cohort of women going through menopausal transition and another from a case-control study of women with and without PCOS.

1.1 Particulate Matter Ambient Air Pollution

Outdoor air pollution, also called ambient air pollution, is a major environmental risk leading to adverse health effects (20). Ambient particulate matter with an aero diameter <2.5 μm ($PM_{2.5}$) is a ubiquitous air pollutant. It comprises a mixture of particles suspended in the air, with local and regional variation. The major sources of PM include motor vehicle emissions, industrial combustion and road dust, and some major chemicals being detected in PM include nitrates, sulfates and carbon (21,22). The history of epidemiology studies which assessed the association between air pollution and human diseases originated from the 1930 Meuse Valley fog event and 1952 London Fog incident, which resulted in numerous deaths and morbidity (23,24). Since then,

although air quality has been improving over the last decades, associations between exposure to current ambient pollution and morbidity and mortality have continued to be documented in the literature (21). A study in 2016 estimated that ambient air pollution contributed to approximately 4.2 million premature deaths worldwide per year (20). Most observational studies and systematic reviews of air pollution and health outcomes' studies have focused on $PM_{2.5}$ as the air pollution exposure, because it is one of the regulated air pollutants with available standards that has shown the most deleterious to cardiovascular health and mortality, and is also the primary component of air pollution (20). Harmful impact of air pollution relates to the increased risk in cardiovascular disease, which has been the leading killer for decades and the nation's costliest chronic disease with an estimated cost of $555 billion in 2016 (1).

1.1.1 Exposure assessment for $PM_{2.5}$

Different exposure assessments were used for estimation of air pollutants in the studies evaluating the association between $PM_{2.5}$ and various health outcomes. The use of those different methodologies of exposure assessment across studies may lead to those inconsistent findings reported when evaluating the associations between $PM_{2.5}$ exposure and its health effect. Early exposure assessment utilized distance of a person's residence from major roadways as surrogate on $PM_{2.5}$ (11,13). More recently, the exposure assessment has evolved from, 1) assigning the nearest monitor's data to individuals living within 10 km of the monitor, 2) estimating city-wide average data and assigning to individuals by their zip codes, 3) utilizing inverse-distance weighting and kriging respectively for modelling exposure data, and most recently 4) using hierarchical models, to obtain spatial estimates of $PM_{2.5}$ exposure for assigning to individuals by census tract, zip code or modeled point estimates.(9,10,12,14,25–29). The recent more reliable modelling

(hierarchical modelling) approach for integrating data from multiple sources allows spatially-varying relationships between ground level measurements and other air quality estimating factors (30). This increased the precision of the measurements and provided an opportunity to extend ground monitoring in areas where monitors are sparse and therefore must be supplemented with other sources (e.g. satellite retrievals of aerosol optical depth and chemical transport models) (31). In summary, those different estimation methods for exposure measurement may yield different results due to the uncertainties of different modellings and exposure misclassification that leads to biased results. It is important to note the method of measurement as it is applied to each study population when evaluating the association between $PM_{2.5}$ exposure and its health effect.

1.2 Cardiovascular Health in Women

Approximately 1 million persons die of heart attack or stroke annually in the U.S. (32). Based on the national health interview survey in 2017, an estimated 28.2 million (11.5%) adults diagnosed with heart disease in the U.S (33). Therefore, it is of significant public health interest to identify modifiable CVD risk factors. The American College of Cardiology and American Heart Association (AHA) published guideline on cardiovascular risk assessment using the atherosclerosis cardiovascular disease (ASCVD) risk calculator in 2019 (34). The guideline includes gender, age, smoking, total cholesterol, high-density lipoprotein cholesterol (HDL-C), systolic blood pressure (SBP) and hypertension to determine 10-year risk of CVD. It is recommended to be used for individuals aged 40-79 years old for ASCVD risk in every 4-6 years (34). Additionally, for individuals aged 20-79 years with no CVD, it is recommended for them to be assessed for traditional CVD risk factors, such as hypertension, dyslipidemia and diabetes (34–

36). Moreover, although the age-adjusted prevalence of heart disease is slightly higher among males than females (12.2% vs.10.0%), women typically develop heart disease several years after men, with greater cumulative risk to an increased CVD and atherosclerosis after menopause (37). Additionally, in the U.S., around 40% of all female deaths are related to CVD (38). This CVD burden, especially among women is a public health concern that needs to be investigated for solutions.

1.2.1 Cardiovascular health and middle-aged women

Heart disease remains the leading cause of death in women in the U.S. (39). There are major differences in the risk, development, and presentation of CVD in women, compared to those in men (40). Traditional atherosclerotic CVD risk factors in women include diabetes, smoking, obesity, physical inactivity, hypertension, and dyslipidemia (41). Additionally, the nontraditional ASCVD risk factors include hypertensive pregnancy, preterm delivery, gestational diabetes, breast cancer treatment, autoimmune disease and depression (41). More recently, early menopause has been added as a sex-specific enhancing risk factor of CVD (42). Previous studies have also reported that there are sex-based differences regarding those listed CVD risk factors, for example, hypertension is less well-controlled in women than men; obesity and smoking affects the CVD risk more in women than in men; and physical inactivity is more common among women than men (41). Furthermore, a review by Garcia et al., 2016, concluded that identifying the modifiable risk factors of CVD is crucial and further study is needed to explore the mechanisms responsible for observed sex difference in those risk factors (41). Because this will help with future interventions and management of CVD risks in women.

Among midlife women (around 55 years old), the CVD incidence rises dramatically. However, a study reported that most women are not aware of sex specific risk factors that occur in midlife, making the midlife women a vulnerable group during their menopause transition (43). Additionally, changes during the menopausal transition can serve as predictors of future CVD risks in postmenopausal women (44).

Taken together, these findings suggest that understanding the interplay between environmental risk factors with unique and specific effect during the menopausal transition will help inform us about the mechanisms of SCA and its impact on CVD health. This can provide an opportunity to conduct preventative screening and increase awareness of potential hazards during this unique period to avoid accentuated CVD risk.

1.2.2 Cardiovascular health metrics in women

The AHA defines cardiovascular health (CVH) as a combination of both physiological and behavioral factors, called the Life's Simple 7 (LS7). LS7 is a set of metrics which includes seven health factors and behaviors including smoking status, physical activity, BMI, blood pressure, total cholesterol, fasting plasma glucose, and diet quality which can be used to assess cardiovascular health (45). Previous study has reported that there is relationship between achievement of LS7 at midlife and lower risk of adverse cardiovascular outcomes (e.g. heart failure) in later life (46). Those factors included in the LS7 have been considered as modifiable factors, and many preventative measures and intervention strategies have been implemented to reduce the CVD burden (47,48). However, so far, other ways to lower the risk for CVD among midlife women, such as reducing the ambient air pollution exposure, are not taken into consideration here (22).

1.3 Atherosclerosis and Coronary Artery Calcium

1.3.1 Atherosclerosis overview

Atherosclerosis, a progressive disease, characterized by the plaque accumulation in the arteries (49). The deposits are made up of lipids, cellular waste products, calcium and clotting materials in the blood (50–52). The atherosclerotic CVD is the major cause of mortality worldwide (51). Additionally, epidemiology data showed that atherosclerosis is more prevalent in the US than Central and South America, and is the underlying cause of 50% of all deaths in westernized society (53). Since atherosclerosis is a predominantly asymptomatic condition, it is difficult to determine the incidence accurately (53).

Potential risk factors for atherosclerosis include smoking, genetic defects, hyperlipidemia, hypertension, high blood pressure, elevated lipids levels, lack of exercise, air pollution exposure and infection (50–52,54). Although not confirmed atherosclerosis risk factors, other CVD risk factors including race, education, and preexisting heart related diseases are related to an evaluated risk of atherosclerosis (21). The etiology of atherosclerosis is complicated and how it starts and progresses are still not clear (54). The most common potential mechanism is due to endothelium damage and plaque accumulation leading to ultimately rupture (55).

1.3.2 Coronary artery calcification overview

The coronary artery calcium score (CAC) plays a crucial role in cardiovascular stratification and cardiovascular event prediction. The CAC score was initially studied by electron beam computed tomography (EBCT) (6) and many investigations have been based on that

technique. Various imaging modalities can be used to assess CAC, and EBCT, the most important noninvasive tool to detect CAC, has been a reliable tool for measuring calcium within the coronary arteries (56). EBCT allows the noninvasive evaluation of coronary arterial atherosclerosis through providing a quantitative measure of coronary calcification. CAC is an active, organized, and regulated process (57).

CAC has been shown to be a better marker for CVD risk prediction than other measures, including carotid plaque and carotid intima-media thickness (CIMT) (58), as it implies the presence of CHD regardless of risk factors or symptoms and is strongly correlated with degrees of atherosclerosis (7,59). The presence of calcium is likely a marker for future cardiac events because it is an indicator of the total coronary artery atherosclerotic burden. A higher CAC score stands for a higher likelihood of atherosclerotic heart disease, indicating higher risk for coronary events. Early detection of CAC in younger subjects has important prognostic impact regarding prediction of future CHD risk (60). Additionally, the CAC score is an independent marker of risk for cardiac events and mortality, and it provides additional prognostic information to other cardiovascular risk markers (61). What's more, finding of the previous study suggest that CAC scores are highly reproducible in middle-aged women (62). The evidence from this study suggest that CAC could be a valid measurement of SCA in middle-aged women for investigations.

Clinically, the coronary calcium scans would be carried out to help guide treatment for patients who have low to moderate risks of heart disease. The most common use of CAC is to screen patients with moderate Framingham risk, while positive CAC scans indicate incremental risk and will alter therapeutic goals (e.g. low-density lipoprotein (LDL), blood pressure) (59). From the public health research point of view, knowing the CAC scores could help motivate people at moderate risk to make important lifestyle changes, for example, modify the outdoor physical

activities based on air quality to reduce the exposure to air pollution. What's more important, we can utilize evidence of harmful effects to advocate change to reduce air pollution at the policy level.

1.3.3 Agatston score overview

Agatston score is a summed score of all calcified lesions considering both calcified area and maximum density of calcification (63). It is the most commonly used calcium scoring and is the gold standard due to its simplicity (56,63). The Agatston score is derived from the product of the area of calcification (mm^2) and a density factor, a measure of maximal density in Hounsfield units (63). Any structure, which had densities ≥130 Hounsfield units (HU) and an area ≥1 mm^2, was segmented as calcified focus and those foci overlying the anatomic site of coronary arteries were considered to represent calcified plaques (63). In each segmented calcified focus, a density score of 1 to 4 was assigned based on its peak density (63). The total Agatston score per patient was calculated by summing the scores of every calcified focus through all of the coronary arteries (63).

1.3.4 Methods for categorization of the CAC presence and progression

The CAC Agtston score in observational study has right-skewed distribution due to many zero values. Because of the skewed distribution, statistically, it is challenge to treat CAC score as a continuous variable or as a categorical variable when building statistical models. In addition, because CAC progression is heavily predicted by baseline CAC, the distribution of CAC progression is always right-skewed. Furthermore, due to many zero scores of CAC, the average

annual rate of CAC progression varies and has wide range from zero to greater than 100. Therefore, other methods for creating or treating CAC progression to be appropriately used in the statistical modelling are crucial to explore. Several observational studies that measured CAC treated it as outcome for building statistical modelling, including the U.S. Multi-Ethnic Study of Atherosclerosis (MESA) cohort study, the population-based Heinz Nixdorf Recall (HNR) study, the Rotterdam prospective cohort study, the Framingham Heart Study (FHS), the Coronary Artery Risk Development in Young Adults (CARDIA) study, and the Jackson Heart Study (60,64–69). We reviewed those studies, as well as some other studies that applied various methods to categorize CAC for evaluating its presence and progression in statistical models, to learn strategies for overcoming the statistical challenges.

1.3.4.1 Categorization of the CAC presence

For CAC presence, the two most common ways to categorize it are: 1) =0 vs. >0; and 2) <10 vs. ≥10 (70,71), as CAC>0 stands for detectable CAC and CAC≥10 stands for CAC presence since patients with CAC<10 confers to a very low risk. Previous studies have used these ways to categorize the CAC presence, such as the U.S. MESA study, the Framingham offspring study, and the Study of Women's Health across the Nation (SWAN) (10,13,72). A few other ways have also been applied to categorize CAC, including treating CAC score as tertile (0 vs. 1-9 vs. ≥10 categorized into 0 vs. 1 vs. 2) to apply multinomial regression (73). The study suggested that CAC score 1-9 group is critical to investigate as separate group, as participants in this group could have real CAC score 1-9 or due to detection error, while CAC=0 is likely to mean there is no CAC and CAC>10 presents a more confirmed CAC presence. Among those studies which treated CAC as a continuous variable, as mentioned above, due to the skewed distribution, the log-transformed CAC was used for those CAC>0, and linear regression was applied (13). Because of many CAC had

zero values, previous studies also added a number (e.g. 1 or 25) to the measured CAC score, and then log-transformed that CAC to apply the linear regression or linear mixed model (10,74).

1.3.4.2 Categorization of the CAC progression

For CAC progression, because inter-scan variability of CAC score is low (~10%), CAC progression estimates are possible (75). The CAC progression that can be determined by the difference between the two CAC measurements or annual progression rate is most strongly predicted by baseline CAC (75). For the annual progression rate, the log-transformed average annual rate of CAC progression ($CAC_{\text{follow-up visit}}$-$CAC_{\text{baseline visit}}$)/(follow-up visit date-baseline visit date)) would be generated. This could reduce the sample size, as participants do not always have repeated measures of CAC. Except calculating the annual progression rate, previous studies have treated CAC progression in several other ways while building statistical models. In a study using the US MESA cohort by Kaufman et al., 2016, the relative progression (average annual rate of CAC change) and the absolute progressions (difference between the CAC measures) were both calculated (10). They then used a ln(Agatston score+25) transformation of the outcome and applied linear mixed model (10). In another study using the SWAN cohort by Wang et al., 2016, they defined CAC progression as present if: 1) baseline CAC = 0 and follow-up CAC score >0, or 2) baseline CAC >0 to <100 and annualized change in CAC score ≥10, or 3) baseline CAC ≥100 and annualized percent change in CAC score ≥10%. And then, they applied logistic regression to model the presence of CAC progression (Yes vs. No) (76).

In summary, although it is statistically challenging to categorize and treat CAC presence and progression in statistical models, previous studies have tested several ways of doing so, which provided strategies for future studies. Viewing CAC as a continuous scale for subclinical precursor to heart disease might be the preferable approach to assess effects of exposures on chronic heart

disease at the population level. As relying on clinical diagnoses or cut-offs as a binary outcome could limit our analysis to an extreme subset of the population and at a point when the disease status may have changed the exposure. However, due to the skewness of the CAC score, it has to be log-transformed to be included in the statistical modelling. Because many participants included in those observational studies were having low to moderate CVD risk so that they are more likely to have zero values for CAC. This therefore leads to statistical issues if treating it as continuous variable and that's why most of the previous studies had to add some values to the observed CAC to be able to log transform those (10). Those modified data makes the evaluation of the association between exposure and CAC less valid and accurate. Since the low values (<10) of CAC are not reliably quantitated sometimes and many women at low or moderate risk of CVD had CAC in this range. The most commonly seen standard practice used by previous studies for treating those low values (including 0) is to categorize it into 0 and compare it with those with CAC≥10 for testing the presence of CAC (77). The implication of this has brought to light many questions in need of further investigation in clinical research, such as what we can base on to impute reliable and valid values for women with low CAC values so that we don't have to exclude or turn them into zeros. Additionally, as those above described studies had been tested and verified, the option of treating CAC as binary variables (e.g. <10 vs. ≥10) for evaluating the association between air pollution and CAC can fully utilize the available data without modifying those, which provides larger sample size (better power) and makes clinical sense regarding the ASCVD risk to do so. Eventually, determining the optimal categorization of CAC would depend on the specific research question, what the chosen population (e.g. moderate risk or high risk), and sample size of the study (e.g. hundreds of or thousands participants).

1.4 Air Pollution and Cardiovascular Disease

1.4.1 $PM_{2.5}$ ambient air pollution and CVD

$PM_{2.5}$ ambient air pollution, coming from many sources (e.g. vehicle, plant, and even windblown dust), can cause acute and chronic cardiovascular conditions (22,78). This is because air pollution levels across the U.S. are periodically high to trigger heart problems (3). Several epidemiological studies suggest that there is an increased risk of CVD effected by fine particles (79–82). There are many different components of $PM_{2.5}$ (e.g. NO, CO), and all their association with health effects have been also studied. $PM_{2.5}$ is the major component of air pollution and has been studied the most. In the most recent AHA statement, a small positive, yet consistent, association between PM and CVD events was reported (22). In addition, several observational studies have reported a positive association between fine particulate air pollution and increased risk for cardiovascular mortality (83,84). The association between $PM_{2.5}$ and CVD could be different in certain subgroups. For example, cause of Metabolic syndromes (e.g. obesity, insulin resistance) have been found to differ the association between $PM_{2.5}$ and cardiovascular risk. In the Women's Health Initiative (WHI) study, a positive association was reported for air pollution and cardiovascular risk among overweight women (BMI>24.8 kg/m^2) vs. leaner women (79). A study in Iran has also demonstrated that $PM_{2.5}$ exposure is associated with worse metabolic insulin sensitivity, indicating a potential increase risk for metabolic syndrome (85). Additionally, different levels of inflammation/hemostatic markers, including homocysteine, fibrinogen, C-reactive protein (CRP), and factor VIII, could also differ the association between $PM_{2.5}$ and cardiovascular risk. Among those markers, CPR has been most studied, as the CRP level increases during inflammatory response to infections. A study using the SWAN cohort found chronic long-term

$PM_{2.5}$ exposure is associated with increased CRP levels, with an even stronger effect in several susceptible subgroups, including low income, high blood pressure and older diabetics (86). What's more, Dabass et al., in 2016 investigated among around 7000 people within a nationally representative sample (NHANES) and determined that long-term $PM_{2.5}$ exposure impact to CVD was significantly greater among individuals with obesity, hypertension, and diabetes (87). In addition, a recent SWAN study provided evidence that the prior year exposure to $PM_{2.5}$ is associated with adverse effects on inflammation (e.g. CRP) and hemostatic pathways for cardiovascular outcomes (88). There is also evidence concerning inflammatory/hemostatic biomarkers (hsCRP, fibrinogen, PAI-1, and tissue plasminogen activator antigen) and the associations with CAC through obesity. A previous SWAN study reported gaseous air pollutants (CO, NO_2, SO_2) and the association with increased thrombotic potential and cholesterol metabolism disruption (89). Additionally, the AHA has recommended physicians and health care practitioners should discuss the CVD risk of exposure to AP although this is currently not a main stream approach (22). An implication of this is the possibility that being able to notice the potential cardiovascular health issues, which is affected by the air pollution, is one way to reduce cardiovascular risk in additional to lifestyle and medication. Furthermore, air pollution has emerged as a modifiable risk factor for the development of CVD (22). According to a recently published finding, exposure to $PM_{2.5}$ is hazardous to health and associated with over 30,000 deaths from cardiorespiratory diseases in 2015 in the U.S (1). Reducing air pollution levels helps with lowering the burden of cardiovascular and respiratory diseases, both short- and long-term (20), and makes a crucial public health impact.

1.4.2 $PM_{2.5}$ ambient air pollution and CVD in middle-aged women

There is a growing body of the negative effects of environmental factors (e.g. air pollution) have a particular impact on women's health, especially among those over 50 (compared to men in the same age group) (90). Supported by several observational studies on CVD risk in women, there is an indication that $PM_{2.5}$ increases their risk of CVD (79). In the Women's Health Initiative (WHI) study, they analyzed 65,000 postmenopausal women from 35 U.S. cities using a prospective cohort study design (79). The long-term average $PM_{2.5}$ ambient air pollution measurements were obtained using the closest monitored data and were assigned to the participants by linking the zip code using the annual average concentration data from 2000 (79). This exposure assessment method as mentioned-above could lead to misclassification of exposure due to the less accurate measures of exposure (compared to those individual level estimates using the advanced modelling approach). This study controlled for a comprehensive list of potential confounding factors, including age, smoking, obesity, diabetes history and hypertension presence (79). They reported that for each 10 $\mu g/m^3$ unit increase in $PM_{2.5}$, the risk of a cardiovascular risk event increased by 24% (Hazard ratio (HR)=1.24; 95% CI: 1.09, 1.41) and the risk of death from cardiovascular disease increased by 76% (HR=1.76; 95% CI: 1.25, 2.47) (79). This estimated risk affected by $PM_{2.5}$ among women from the WHI study was larger than those reported in previous well-known general population studies in the U.S, including the Six Cities Study (76% increase for WHI vs. 28% increase) (79,91). The Six Cities Study was a prospective cohort and the above mentioned study, which conducted by Laden et al., 2006, used average $PM_{2.5}$ over the entire follow-up (1974-1998) and average $PM_{2.5}$ in 1979-1988 for their main exposures (91).

The finding of previous study suggest that women are at increased risk of CVD, overall mortality and cardio-metabolic disease after menopause (92). Additionally, the results of an

investigation have addressed the ways in which the vulnerable period of accelerating cardiovascular disease risk, when women are transitioning through menopause, may be more than just change of chronologic aging (93). Taken together, these findings may indicate that the menopausal transition may have additional effect resulting in greater cumulative risk to their cardiovascular system. Several studies used the SWAN cohort have also addressed the vulnerable period for women, as their transition through menopause, which is related to biological changes (e.g. change in endocrine, and other physiological changes, such as changes in lipids and lipoproteins) (72,88,94,95). This vulnerability may act to not only increase risk for atherosclerosis among this population particularly but also augment the impact of air pollution on the progression of atherosclerosis. In other words, while traditional risk factors are more prevalent in women during menopausal transition, the greater CVD risk may be attributed to the synergistic interaction between air pollution and menopause that emphasize CVD risk and contribute to accelerated atherosclerosis.

Therefore, it is important to look at association between air pollution and cardiovascular health in this sensitive to CVD risk population, middle-aged women. If this is proven to be true, consideration should be given to educating middle-aged women without CVD who are at high risk (e.g. during menopausal transition, individuals have features of metabolic syndrome, e.g. women with PCOS). Another motivation is that if we can measure these types of effects of air pollution within women undergoing these transitions, it is strong evidence that air pollution is having adverse effects on people of all ages. It makes sense to single them out for the purposes of a valid study design and higher statistical power.

1.4.3 Biological mechanisms: $PM_{2.5}$ ambient air pollution and CVD

Observational and experimental investigations support the association of $PM_{2.5}$ to CVD. However, the biological mechanisms for $PM_{2.5}$ health effects are not clearly understood. The proposed potential biological mechanism linking the air pollution and CVD has been summarized by Brook in 2010 America Heart Association (AHA) statement (See **Figure 1.1**) (22). In this AHA statement, a small positive, yet consistent association between PM and CVD events was reported (22). Additionally, several studies have reported a positive association between fine particulate air pollution and increased risk for cardiovascular mortality and progression of atherosclerosis (10,83,84). The biological mechanism behind the PM-mediated enhancement of atherosclerosis may be due to the pro-oxidant and pro-inflammatory effects (54). The most common theories regarding the mechanism are inflammatory response and oxidative reaction. At cellular level, cell types, including lung epithelial, endothelial cells, and cardiomyocytes, have shown to respond to in vitro PM exposure with elevated ROS levels and oxidative stress (96–98). Furthermore, it has been demonstrated in animal studies that long-term exposure to ambient $PM_{2.5}$ leads to both endothelial dysfunction and accelerates the progression of atherosclerosis, especially among mice deficient in apolipoprotein E or low density lipoprotein receptor, which develop advanced atherosclerotic lesions (99). Those indicate that air pollution may accelerate the development of coronary atherosclerosis and progression of atherosclerosis over time, through one or more of those pathways mentioned above.

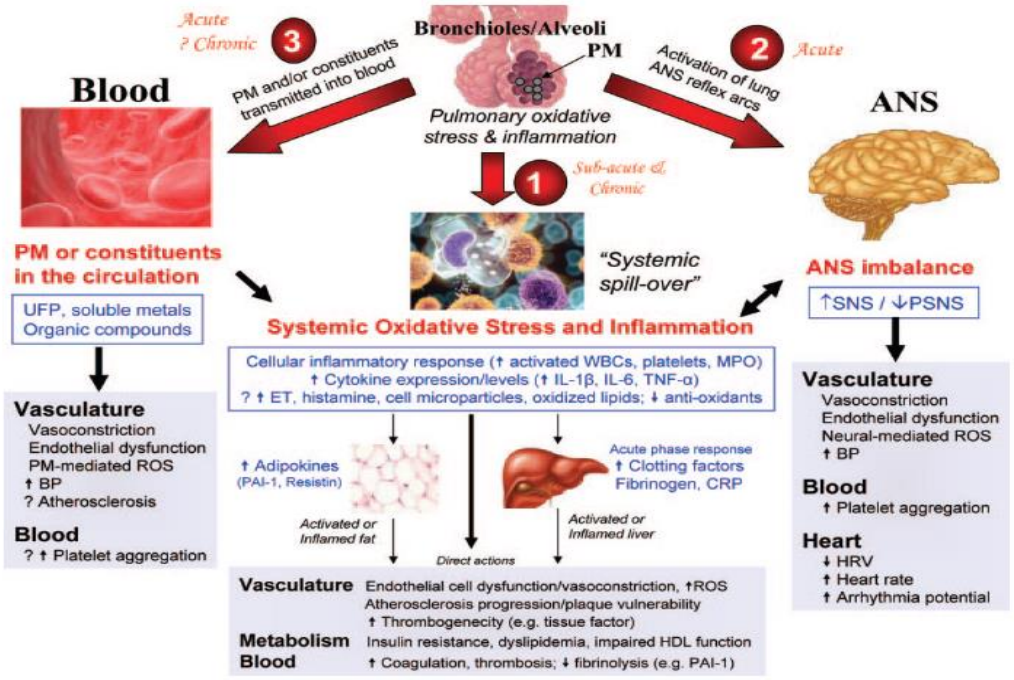

Figure 1.1 Biological pathways linking PM exposure with CVDs[a]

Inhalation of the tiny particles (e.g. $PM_{2.5}$) deep into lungs and blood circulation through the alveolar-capillary membrane may stimulate an inflammatory response (e.g. increased levels of interleukin (IL)-6, nitric oxide (NO)) and decrease cell viability in human coronary artery epithelial cells, which impacts vascular health, further hardening arteries (100). Stimuli such as smoking or air pollutant exposure may initiate the development and progression of calcification through the death of smooth muscle cells (SMC), which could be the driving force for early microcalcification (56). This is then followed by infiltration of macrophages into the lipid pool, which undergoes cell death and calcification leading to the inflammation (101). In addition, reactive oxygen species (ROS) regulate the activity of redox-sensitive signaling pathways and impact diverse aspects of vascular biology (2). Inhalational exposure to air pollution triggers not only inflammation but also major ROS generated in air pollution exposure, which affects NO

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

release, leading to endothelial dysfunction, with detrimental effect on vascular cells (2–5). Taken these together, the commonly proposed physiological pathways linking air pollution and atherosclerosis are through: 1) systematic and pulmonary inflammation and oxidative stress; 2) vascular endothelial dysfunction; and 3) atherothrombosis (100).

1.5 Menopausal Transition, Polycystic Ovary Syndrome and Cardiovascular Risk

1.5.1 Menopausal transition, menopause, and cardiovascular risk

1.5.1.1 Menopause

Menopause is a stage in life when women stop having monthly period, and it normally happens within one year after the final menstrual period (FMP) (102). In western populations, the average age at menopause is 51 years, but the range of age at onset is wide, with most women experiencing menopause between late 40s and 50s (103–105). Women who have their ovaries surgically removed experience surgical menopause when those surgeries occur. Menopausal status is commonly defined by changes in menstrual bleeding, which is a proxy for changes in sex hormone levels (e.g. estradiol, progesterone, or testosterone) (38). Based on the 2011 Stages of Reproductive Aging Workshop (STRAW), STRAW+10 criteria, the adult female life can be divided into late reproductive, early menopausal transition, late menopausal transition, early postmenopause, and late postmenopause (106).

1.5.1.2 Menopausal transition

The menopausal transition is the period when the levels of hormones produced by the aging ovaries fluctuate, resulting to irregular menstruation and hot flashes (16). This period encloses both chronologic aging and reproductive axis aging of the menopausal transition (16), and can begin several years before a woman's last period. There are numerous observational studies of longitudinal cohorts conducted among women transitioning through menopause in relation to CVD, which have contributed significantly to our understanding of the relationship between the menopause transition and CVD risk (93). By following women over the menopausal transition, researchers were able to understand the difference between chronologic aging and ovarian aging effects in relation to CVD risk. One of these studies, the SWAN was a longitudinal study that followed a cohort of women from premenopausal years through the menopause transition and into postmenopause, was designed to address the relative contributions of chronologic and reproductive aging to women's CVD risk and to characterize the biological and psychosocial antecedents and sequelae of the menopausal transition (16,93). In a recent progress report of the SWAN, they summarized the contributions of the menopause transition to women's cardiovascular health based on the previous findings of the SWAN, and those include increased lipids level (e.g. LDL-C), adverse changes in vascular remodeling and metabolic syndrome (93).

1.5.1.3 Menopausal transition, final menstrual period and cardiovascular risk

Although the CVD burden has declined over the past decades, it remains the leading cause of death especially among menopause women, who had an increasing CHD risk (16,107). Premenopausal women are relatively protected from CVD risk, compared with men in the same age group. However, this sex-specific difference in CVD risk is reduced after menopause (41). Additionally, studies have been conducted to focus on women transitioning through menopause to

explore what changes occurred during that period diminished CVD protective effect of women after menopause. So far, the menopausal transition and its association with CVD risk have been investigated by many studies and researchers have pointed out that the menopausal transition is more than a dramatic decline in hormone level (108,109).

Many other factors converge at the same time and create multiple cardiovascular risk factors within the system. It is at this point that $PM_{2.5}$ may add additional insult and may work to incur greater health effects. Taken together, these findings suggest that understanding the interplay between hormone dysregulation during the menopausal transition can inform us about the mechanisms of SCA (93,95,102,110–112). What's more, researchers have noted an earlier age of menopause is associated with increased risk of CVD and cardiovascular risk factors (e.g. higher HDL, hypertension) (113). Early age at menopause results in endogenous estradiol fluctuation, however, other changes occurring during menopausal transition have been linked to CVD risk, such as menopause stages of perimenopause stage and vasomotor symptoms. Additionally, hormonal changes occur across the menopausal transition, with various patterns detected around FMP (e.g. some women experienced patterns of estradiol decline, or follicle-stimulating hormones rise over the menopause transition, but not all women) (93), making the time around FMP crucial to study. In addition, the FMP can be used to define menopause and signifies depletion of ovarian follicular reserve and endogenous estradiol (114). More specifically, estradiol (E2) and follicle-stimulating hormones (FSHs) changed dramatically over the menopausal transition. E2 commonly decreased from 2 years before the FMP to approximately 2 years after the FMP. The levels of E2 stables after 2 years of the FMP. What's more, FSH increased across the menopausal transition with a gradual increase from 7 years before the FMP to 2 years before the FMP. The levels of FSH dramatically increased from 2 years before the FMP to 2 years after the FMP, with a stabilization

2 years after the FMP. (93,115) The SWAN study has shown that not all midlife women experienced one pattern of E2 decline or FSH rise over the menopausal transition, and the heterogeneous patterns were observed across the SWAN women (e.g. obese vs. non-obese midlife women) (116). These findings add substantially to our understanding of the dynamic hormone changes occurring during the menopausal transitions (E2 levels dropping, follicle-stimulating hormones (FSH) rising, etc), and support the idea that we may adjust for factors (e.g. E2) for evaluating the role of menopausal transition to see how it might affect the tested association (e.g. $PM_{2.5}$ and CAC).

Previous studies reported that the timing of women's FMP has shown to be correlated with cardio-metabolic risk (93,95,110,111). During the menopausal transition, women experienced adverse changes in their lipids and metabolic syndrome risk. The low-density lipoproteins cholesterol (LDL-C) increased around FMP, which is associated with greater risk of plaque presence after menopause (95). The high-density lipoproteins cholesterol (HDL-C) increased to be the highest during the late peri- and early postmenopause stages (110). However, the slightly increased or remain stabled HDL-C during the menopausal transition may not be always protective to cardiovascular health (93,111). Additionally, inflammatory markers, responding to the fluctuations during menopausal transition, include adverse changes in levels of C-reactive protein (CRP) and hemostatic markers (e.g. tissue-type plasminogen activator antigen (TPA), and plasminogen activator inhibitor Type 1 (PAI-1)). Those changes, such as change in CRP across the menopausal transition may not clearly be attributed to hormonal changes though (112). As a study concluded, changes in blood pressure, insulin, glucose and BMI during midlife were not associated with the menopausal transition, instead, those changes are more relevant to the chronological aging (93,117–119). Those studies have gone some way towards enhancing our

understanding of the potential pathways linking the menopausal transition and cardiovascular risk through cardio-metabolic factors addressed above.

In summary, studies have documented dramatic changes in endogenous sex hormones and adverse changes in lipids and lipoproteins, and vascular remodeling over the menopausal transition (16,44,94,120,121), as described above. These changes together can increase women's risk of developing CVD later in life. As previous studies have addressed, menopausal transition is more than a dramatic decline in hormone levels (108,109), and this time window of menopausal transition or time close to the final menstrual period can be a time of accelerating CVD risk, leading to women's cardiovascular events during their postmenopausal stage. Therefore, monitoring women' health during their midlife is critical to find ways of applying intervention reducing their CVD risk at midlife. Additionally, for population studies of air pollution, women during menopausal transition offer a unique opportunity to quantify subclinical effects of $PM_{2.5}$. Results are important for this subpopulation, but also important for understanding overall risks in the whole population. Exposure to $PM_{2.5}$ is likely having similar effects in all people, but they are not as easily measured as compared to women who are undergoing this transition.

1.5.2 Polycystic ovary syndrome and cardiovascular risk

PCOS is a complex disorder of the endocrine system characterized by chronic anovulation, hyperandrogenism, and insulin resistance and is a common endocrine disorder of reproductive-aged women (122,123). Previous studies suggest that PCOS affects 6-10% women, and prevalence may actually be twice as high (124,125). This translates to over 17 million women in the US alone (125). PCOS usually develops at puberty and is mostly inherited (genetic disorder) (126). As PCOS is characterized by insulin resistance and inflammation, there may be a mix of genetic and

environmental factors have been implicated (127,128). Clinically, PCOS is diagnosed in women with ≥2 of the features, including: 1) hyperandrogenism, 2) ovulatory dysfunction, and 3) polycystic ovarian morphologic features (122). Clinical symptoms of PCOS include acne, amenorrhea or oligomenorrhea, hirsutism, infertility, and mood disorders (129). In a large-scale epidemiological study (Pittsburgh based study), diagnostic criteria of PCOS was established if there was a history of chronic anovulation in association with either (1) evidence of clinical (hirsutism) or biochemical hyperandrogenism (total testosterone level > 2.0nmol/L) or (2) a luteinizing hormone: follicle-stimulating hormone ratio (LH:FSH) > 2.0 (130).

1.5.2.1 PCOS and CVD risk factors

It is most commonly diagnosed during women's reproductive lifespan (20-30 years old) when there is a wish to conceive, and their CVD normally manifests 30-40 years later. The association between PCOS and CVD therefore has been difficult to study, because of the long latency period of CVD in women with PCOS. Large long-term observational studies of well-characterized women with PCOS on this topic are rare, as it requires very long time of follow-up of women with PCOS from the premenopausal reproductive age until menopausal range (≥50 years) when there is greater cumulative risk to an increased CVD. The Women's Ischemia Syndrome Evaluation (WISE) study and a retrospective cohort study in the United Kingdom are two of those large-scale epidemiological studies that have investigated the long-term sequelae of PCOS and the results of the morbidity and mortality endpoints (131,132). The WISE study from the U.S. with 295 postmenopausal women enrolled and 25 of the women (8%) were defined as having clinical features of PCOS. When compared with women without PCOS clinical features, women with PCOS had more (not statistically significant) angiographic coronary artery disease (CAD) but the cumulative 10-year mortality was similar (28% vs. 27%). This longer-term follow

up of a relatively small cohort of postmenopausal women with suspected ischemia concluded that clinical features of PCOS are not associated with CAD or mortality (132). Another long-term study in the United Kingdom above-mentioned with 345 women diagnosed with PCOS and 1060 age-matched controls reported that women with PCOS is associated an increased stroke related mortality (OR=3.4; 95% CI: 1.2-9.6) but not CHD mortality even after adjustment for BMI, compared with women without PCOS (131). Additionally, this study by Wild et al., 2000 also found association between PCOS and increased cardiovascular risk factors, including hypertension, diabetes, hypercholesterolemia, hypertriglyceridemia, and an elevated waist-to-hip ratio (131). Except those listed major risk factors, PCOS is associated with high androgen levels and it is possible that there is an important link between menopause biology and PCOS biology regarding to their relationship with CVD risk. However, in a Rotterdam study they found that postmenopausal high androgen levels were not associated with an elevated risk for CVD among general population (133). This suggested that the effect of androgens on the cardiovascular system might not be fully elucidated yet to allow us look at this potential pathway among midlife women with PCOS.

In summary, the following conclusions can be drawn by reviewing those above-mention studies and a few other studies of women with PCOS. They are: 1. there is an association between PCOS and CHD, and PCOS has been studied as a risk factor of the increased risk of CVD (131,132,134). 2. the major CVD risk factors appear to be more common among women with PCOS, including, obesity (increased central adiposity or BMI, or waist-to-hip ratio), insulin resistance (hyperinsulinemia), dyslipidemia (elevated LDL or triglycerides), and type 2 diabetes (131,132,134–136). Those will serve as a base for future studies to consider when assessing the

association between other independent variables (e.g. air pollution) and cardiovascular conditions among women with PCOS.

1.5.2.2 PCOS and CAC

Women with PCOS represent the largest subgroup of the female population at high risk for early SCA and subsequent clinical CVD (137). In addition, a growing number of epidemiologic studies have reported women with PCOS have greater amounts of coronary calcification and endothelial dysfunction as well as increases in carotid intima medial thickness (73,130,138,139), which is consistent with an crucial sex steroid hormone effect. In one of the largest groups women with PCOS case-control study by Talbott et al., the study reported that women with PCOS had significantly lower levels of estradiol, higher apolipoprotein B levels, and higher triglyceride levels (73,138), which are also the risk factors of CAC. The study also concluded that there was a significantly higher rate of detectable CAC among women with PCOS compared with controls (54% vs. 24%, $p< 0.05$) (73). Additionally, a cross-sectional study using a group of premenopausal women (aged 30-45 years) has concluded that women with PCOS have a greater prevalence and extent of coronary calcification than in obese or non-obese women with similar age women and obesity increases the risk of CAC in those premenopausal women with PCOS (140). Furthermore, studies have also reported that among women with PCOS, BMI is a significant predictor of the increased CAC risk (138,140,141). Those above-mentioned findings suggested that the increased risk in CAC among women with PCOS could be due to those factors/profiles that were different in women with PCOS compared with those without PCOS, including: elevated LDL-C, decreased HDL-C and hyperinsulinemia (138,140,141). Taken together, women with PCOS could be targeted for prevention of cardiovascular disease and atherosclerosis (CAC), such as making them aware of their potential higher risk of atherosclerosis so that they may change their lifestyle or

reduce their environmental exposure as prevention strategies. To our knowledge, no study so far has conducted to assess the association between $PM_{2.5}$ and CAC among women with PCOS to determine whether women with PCOS is even more sensitive to CAC risk with the additionally effect of $PM_{2.5.}$

1.6 Association Between Air Pollution and Atherosclerosis

Experimental exposure and animal studies have provided evidence on the association between long-term $PM_{2.5}$ exposures and progression of atherosclerosis under the controlled experimental environment (142,143). Observational studies in human have reported inconsistent results regarding to the association between $PM_{2.5}$ and atherosclerosis (144–146). Carotid intima media thickness (CIMT) were evaluated the most as measure of subclinical atherosclerosis to explore its association with $PM_{2.5}$ and those investigations have yielded mixed results (8,12,83,144–148) In a meta-analyses study by Akintoye et al., 2016 on this topic, they reported evidence to support the association between $PM_{2.5}$ exposure and CIMT, with considerable heterogeneity (I^2=83%) among the included eight studies which conducted in 2008-2014 (149). For each 10 $\mu g/m^3$ increase in $PM_{2.5}$ exposure, they reported a 22.52 μm increase in CIMT, though the association they reported did not reach statistical significance (p=0.06) (149). Another measure of atherosclerosis is by CAC, which is a better predictor to the CVD risk than CIMT. In the same meta-analysis study by Akintoye et al., 2016, they reported that based on the three studies they identified, there is no evidence of an association between $PM_{2.5}$ exposure and CAC risk (8,11,12). Although three studies evaluated the association between $PM_{2.5}$ exposure and CAC presence have been included in this meta-analyses, two out of those three studies used the same cohort, the US

MESA study cohort (8,12), and because of this small number of the included studies, they did not test for heterogeneity. Additionally, for including only two study cohorts assessed this association in their meta-analysis, the authors concluded that they have been underpowered to find a meaningful association as they found a low number of studies evaluated the association between $PM_{2.5}$ and CAC (149). However, after we searched for literature on this topic, we identified seven studies that assessed the association between $PM_{2.5}$ exposure and CAC (8–14), including both CAC presence and CAC progression. Except one study was published in 2019, all other six studies were published before that meta-analyses, and some reported differing findings from the earlier meta-analysis (10,13,14). Therefore, this meta-analysis by Akintoye et al., 2016 missed to include three studies; additionally they did not review studies evaluated CAC progression and its association with $PM_{2.5}$. These suggest that a more thoroughly review on the association between $PM_{2.5}$ exposure and CAC will be needed with including all the published studies on this topic to further explore what previous studies found on whether the association exists. We therefore conducted a narrative review on this topic and presented our findings in the paper 1 of this dissertation. In the next section, we will provide some brief overview and summary on those included seven studies as introduction, and then discuss more thoroughly in details in our paper 1. Among those observational studies, which assessed the association between ambient air pollution ($PM_{2.5}$) and CAC, the mean level of $PM_{2.5}$ exposure among the US and Germany studies ranged from 13.7 to 22.8 $\mu g/m^3$; except the Chinese study, which has the average level 70. $\mu g/m^3$ (SD=20.0) (8–12,14). Overall, those were only four populations (the Germany study, the US MESA study, the US Framingham Offspring study, and the Chinese study) studied and two of them showed positive association between $PM_{2.5}$ exposure and CAC, whereas the remaining five

did not provide such evidence (8–14). Both of these two studies were conducted more recently and employed spatiotemporal exposure assessment (10,14).

1.6.1 Association between $PM_{2.5}$ exposure and CAC progression

For all of the seven studies evaluated the association between $PM_{2.5}$ exposure and CAC, except the most recent U.S. MESA study by Kaufmann, et al., 2016 and the Framingham Offspring cohort study by Dorans et al., 2016, others assessed the association (for CAC presence) using a cross-sectional study design (8–14). The association between air pollution and progression of subclinical atherosclerosis through repeated CAC measurements by Agatston has been evaluated by only two studies so far (10,13). The longitudinal study, by Kaufmann et al, 2016, using the MESA cohort (N=6795 US people, 45-84 years old) reported a positive association between $PM_{2.5}$ and CAC progression (Agatston unit yearly change per IQR $PM_{2.5}$ (5μg/m^3) increase: 4.1 (95% CI: 1.4 to 6.8)) (10). This is currently the first longitudinal study demonstrated a positive association among the four studies all used MESA cohort assessed air pollution and CAC association (10). There was yet another longitudinal study conducted using the Framingham Offspring cohort who were the offspring from the original Framingham Heart study conducted in Framingham, Massachusetts in 1948 (150,151). In that Framingham Offspring cohort, Dorans et al (2016) utilized the measured CAC ≤2 times from 2002 to 2005; and 2008 to 2011 and assessed associations of residential fine particulate matter using the 2003 average $PM_{2.5}$ exposure (spatiotemporal model) with detectable, presence, extent or progression of CAC (13). They did not observe association between $PM_{2.5}$ exposure and any CAC.

1.6.2 Association between $PM_{2.5}$ exposure and CAC presence

There are several cross-sectional studies conducted on this topic. Inconsistent or non-statistically significant findings were reported by the cross-sectional studies (the Germany study, the US MESA study, and the Chinese study) related to $PM_{2.5}$ and CAC presence association (8,9,11,12). The only cross-sectional study reported positive association between long-term $PM_{2.5}$ exposure and CAC was the Chinese study by Wang et al, 2019 (Agatston unit % increase per $PM_{2.5}$ IQR increase (30 $\mu g/m^3$): 27.2; 95% CI: 10.8 to 46.1%) (14). The first investigation to consider residential exposure to traffic and coronary atherosclerosis was conducted by Hoffman et al, 2007 using the German Heinz Nixdorf Recall Study cohort (N=4494) (11). They reported that $PM_{2.5}$ exposure was not associated with higher CAC in all subjects (12.7% higher CAC per IQR increase in $PM_{2.5}$ <3.91 $\mu m/m^3$>, 95% CI: -5.6, 45.5) (11). Diez Roux et al (2008) was the first group of investigators to consider long-term exposure to $PM_{2.5}$ exposure and SCA using the MESA population (12). They used a multifactor set of outcomes including CAC (n= 5,172) and the 20-year prior to $PM_{2.5}$ assessment (1982-2002 or the baseline exam) imputed mean $PM_{2.5}$ data, which obtained using a spatial-temporal model, and the estimated mean 2001 $PM_{2.5}$ exposure data (12). Neither of the $PM_{2.5}$ exposure measurements they used were associated with CAC overall. Another study used the MESA cohort by Sun et al, 2013, further considered total $PM_{2.5}$ mass and $PM_{2.5}$ components (sulfur, elemental carbon, silicon and organic carbon) when evaluating the association with CAC. Community monitors deployed for the MESA study starting from 2007-2008 were used to estimate exposures at baseline (2000-2002) and none of the $PM_{2.5}$ components was associated with risk of CAC (8). Kim et al (2014) within MESA also evaluated chemical constituents within fine PM (sulfur, carbon, silicon and organic carbon) and their associations with CAC, using the

different exposure monitoring data and modelling approaches than Sun et al., 2013 did and found no evidence of a CAC increase in relation to the modeled $PM_{2.5}$ exposures (9).

1.6.3 Association between $PM_{2.5}$ exposure and CAC among women

Observational studies suggest that the increased CVD risk from fine particles may be gender specific with postmenopausal women being more susceptible (79). In a recent study assessed association between $PM_{2.5}$ exposure and CAC, there is a study further evaluated this association among the sub-population of women, by Wang et al, 2019 (14). This study conducted sensitivity analyses among postmenopausal women (N=1732 out of 3790 women, around 45.7% were postmenopausal women). Wang et al (2019) reported that the positive association of $PM_{2.5}$ exposure and CAC was significant and much stronger among the subgroup of women compared with general population of their study (34.5% Agatston unit increase per 10 $\mu g/m^3$ increase in $PM_{2.5}$ exposure; 95% CI: 5.8% to 70.9%), adjusted for age and other confounders (14). This finding suggests that there is a stronger effect after menopause for $PM_{2.5}$ exposure to CAC. Although, the remaining studies assessed association between $PM_{2.5}$ exposure and CAC neither adjusted for menopause status nor reported gender as an effect modifier for evaluating the association between $PM_{2.5}$ exposure and CAC. Women may be more susceptible to CAC risk than men do, and this could be due to various reasons, including life style choices, environmental exposures, and risk of CVD (e.g. diabetes, hypertension, obesity, and inactivity) which are seen more frequently in postmenopausal women than in men. Although the investigations conducted so far do not answer why postmenopausal women's CAC risk affected by $PM_{2.5}$ might be higher. In addition, the biological mechanisms for $PM_{2.5}$ health effects that may be causing the observed gender effects are less understood. For exploring those potential mechanisms, studies focusing on subpopulation

of women with unique physiological profiles could be helpful, such as women going through menopause transition as they have unique profiles of hormone and inflammation markers' levels; and women with PCOS due to their increased risk in presenting with insulin resistance and hyperinsulinemia (152–154). In the section 1.5.5, we will review the studies related to the intermediate role of the menopause transition and/or PCOS to the association between $PM_{2.5}$ exposure and CAC, to provide information on how can we evaluate those factors in the investigation of assessing the association between $PM_{2.5}$ and CAC.

1.6.4 Association between $PM_{2.5}$ exposure and PCOS in middle-aged women

To our knowledge, there is no study investigating the association between $PM_{2.5}$ and CAC among women with PCOS. Evidences supporting the study of the association between $PM_{2.5}$ and CAC among middle-aged women with and without PCOS will be presented in this section. First, a study pointed out that when PCOS women reach the menopause, the second metabolic "insult" may be associated with a greater cumulative risk to an increased CVD and atherosclerosis (73). With the potential biological mechanism proposed through the inflammation and oxidative stress in disease progression in endometriosis (shares similarity with PCOS, e.g. both can result in irregular bleeding) and infertility (PCOS could cause infertility) (22), it will be crucial to assess the association between air pollution and CAC risk among women with PCOS. As there is little information available on their (women with PCOS) physiological changes during midlife, which may make the association between $PM_{2.5}$ exposure and atherosclerosis different in those women with PCOS than in women without. For example, previous study found that PCOS women have substantially greater amounts of CAC as well as lower vasodilation, a measure of endothelial function (73). It is possible that the effect of $PM_{2.5}$ on women with PCOS makes them more

vulnerable to an increased risk of CAC during midlife. Study the unique population, women with PCOS, provides a natural experiment for evaluating of the hormonal abnormalities effects and other physiological changes and $PM_{2.5}$ on cardiovascular risk. To understand the potential impact of PCOS on the association between $PM_{2.5}$ and CAC among midlife women, it would be of interest to compare this association among middle-aged women with and without PCOS.

Additionally, although the causes of PCOS is still not well understood, several investigations have considered environmental factors, such air pollution might be associated with it (155,156), which could be the interplay effect of genetic and environmental factors. As mentioned earlier, no study has conducted so far on assessing the association between $PM_{2.5}$ and CAC among women with PCOS. A systematic review evaluated the effect of $PM_{2.5}$ to some symptoms that related to PCOS. Although the outcome measure is not PCOS, PCOS is one of the most common causes of infertility; and in the systematic review, they reported that there is a potential relationship between air pollution and infertility. This meta-analysis did not provide pooled mean estimates or heterogeneity I-squared statistics for their overall findings, which could be due to the small number of the studies they were able to identify. They did address that it is important for future studies to estimate the association between $PM_{2.5}$ exposure and women's health among PCOS population. As a large number of women at risk of PCOS (10% for PCOS), implement interventions for reducing the disease burden among this population will be crucial.

Taken together, those findings of the above-mentioned studies have shed some light on the potential association between $PM_{2.5}$ and PCOS. This serves as a base for future studies and illustrates the importance of exploring the association between $PM_{2.5}$ and CAC among middle-aged women with PCOS to compare to those without PCOS.

1.6.5 Consideration for potential intermediators

In this section, we will present a summary statement based on the findings from those above mentioned studies we reviewed that investigated the associations between air pollution/$PM_{2.5}$ and the potential intermediary variables (confounder, modifier or mediator, e.g. CRP, lipid, E2), and between those intermediators and CAC among middle-aged women.

In summary, the possible pathways linking the association between $PM_{2.5}$ and CAC we may explore in the future studies include: 1) $PM_{2.5}$ exposure that affects inflammation markers (e.g. CRP) and that affects CAC; 2) $PM_{2.5}$ exposure that affects lipid levels in perimenopausal women, which may affect CAC development; and 3) $PM_{2.5}$ exposure that increases risk for cardiovascular risk factors (e.g. insulin). As our review implies that those factors could be affected by $PM_{2.5}$, as well as are associated with higher risk of CVD (CAC). The longitudinal observational cohort with those variables available will be helpful to test those potential links. Additionally, our reviewed studies' enhance our understanding on what cofounders we may adjust for when assessing the association between air pollution and atherosclerosis and what intermediators we can evaluate as mediators or effect modifications. We have to keep in mind that when we assess the association between $PM_{2.5}$ and CAC among middle-aged women, the reasonable confounders are those that related to both $PM_{2.5}$ exposure and CAC, such as the residential address or social economic status. Other intermediary variables, such as inflammation markers, hormone levels (E2), would more likely to be mediators or effect modifiers that we can evaluate. Because of the various biological changes occurring during women's midlife, it is important to explore how those changes may play a role on association between $PM_{2.5}$ and CAC during women's midlife. Future research needs to be done to establish the association, in the meantime, to explore the reasonable intermediary variables for understanding the effect on the association.

2.0 Specific Aims

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

Aim 1. To review the results of the studies of long-term $PM_{2.5}$ and SCA, measured as CAC in the general population with a focusing on the exposure measurement methodologies and results of the studies of $PM_{2.5}$ and CAC.

Aim 2. To evaluate the association between exposure to long-term $PM_{2.5}$ and CAC among middle-aged women of the SWAN heart and to determine if the menopausal transition (measured by menopausal status and time since FMP) modifies this association.

Hypothesis: Exposure to long-term $PM_{2.5}$ is associated with an increased CAC presence and progression among middle-aged women of the SWAN heart study and the association is stronger during the menopausal transition.

Aim 3. Within a unique population of women with PCOS, we will assess the association between long-term $PM_{2.5}$ exposure and CAC and further assess whether this association varies by PCOS using CHARMIII study.

Hypothesis: Exposure to long-term $PM_{2.5}$ is associated with an increased CAC presence among middle-aged women of the CHARMIII study and the association is stronger among women with PCOS.

3.0 An Overview of the Association between Long-Term $PM_{2.5}$ Exposure and Subclinical Atherosclerosis, in the General Population and in Middle-Aged Women: is the Menopausal Transition a Critical Window?

3.1 Abstract

Background and Objective

Less is known about the long-term effects of air pollution (AP) on subclinical atherosclerosis (SCA) as the condition takes time to develop and may require better-designed longitudinal studies to capture the disease progression. Observational studies that have assessed the associations between long-term fine particulate matter <2.5μm ($PM_{2.5}$) AP exposure and SCA measured by coronary artery calcification (CAC) have shown mixed results. These investigations have shown that women in midlife could be a population at risk for an increased effect of AP, but none of these studies were organized or developed specifically with this focus. Therefore, the review is aimed at evaluating to what extent midlife women were included and evaluated to determine the effect of $PM_{2.5}$ and CAC. This review describes the exposure measurement methodologies and results of the studies of $PM_{2.5}$ and CAC with this group in mind and culminates in making commendations for future research regarding women in midlife, a potential "at risk" population. We also review additional studies assessing the association between carotid intima medial thickness (CIMT) and $PM_{2.5}$, focusing on evaluating whether the interactions by gender or age were assessed.

Methods and Results

Seven studies representing four cohorts investigated the associations of $PM_{2.5}$ exposure and CAC were identified through PubMed, and information on 1) design of each investigation; 2) population at risk /clinical measures; 3) exposure assessment methodology; 4) timing of exposure measures and the definitions of long-term exposure; and 5) specific results of the association of $PM_{2.5}$ and CAC were provided. Additionally, five studies on the association of $PM_{2.5}$ exposure and carotid intima-media thickness (CIMT) with a focus on assessing the age and gender differences were also included.

Two out of four CAC related cohorts (the US Multi-Ethnic Study of Atherosclerosis (MESA) cohort and the Coronary Atherosclerosis Disease Early Identification and Risk Stratification by Noninvasive Imaging (CREATION) Chinese cohort) reported results that support a relationship between long-term $PM_{2.5}$ exposure and CAC development and progression and the remaining two cohorts reported largely null findings. Several different methods for exposure assessments were used for $PM_{2.5}$ estimation in the studies evaluating the association with CAC. The use of different methodologies of exposure assessment across studies may have led to the variations in the reported findings. Two of our reviewed studies support a potential stronger effect among women or among women in postmenopausal status for $PM_{2.5}$ exposure to CAC, though the studies evaluating the association between long-term $PM_{2.5}$ exposure and CIMT have not reported gender or age as a statistically significant effect modifier to the association. The studies we reviewed were not able to assess the menopausal transition as an effect modifier for evaluating the association between $PM_{2.5}$ exposure and SCA.

Conclusion

Our review of previous observational studies found that there is equivocal evidence with some, but not all, studies reporting associations between $PM_{2.5}$ exposure and CAC presence or progression among the general population. This is an important gap because previous studies suggest that women are particularly vulnerable to SCA risk as they transition through menopause. We offer approaches to enhance the study of long-term effect of AP on SCA for future investigations.

Keywords: Particulate matter, Air pollution, Long-term exposure, Subclinical atherosclerosis, Coronary artery calcification, Midlife women

3.2 Introduction

Air pollution is a well-established public health problem. Most notably, particulate matter with an aerodynamic size of <2.5μm ($PM_{2.5}$) and <10μm (PM_{10}) has been studied extensively for over thirty years (157). The association of $PM_{2.5}$ and increased risk of acute myocardial infarction has been well documented by the application of both time series and case crossover analyses which are effective approaches to capture acute effects(17–19). However, less is known about the long-term or chronic effects of AP on the cardiovasculature. This requires a longitudinal or retrospective cohort design in which exposure has accumulated over a large span of time (73,158). Subclinical atherosclerosis (SCA) is one example of a cardiovascular outcome relevant to $PM_{2.5}$ exposure, takes decades to develop and is difficult to assess. Given the availability of historic AP data, average cumulative AP exposure (a year or more) can be estimated and mapped to an individual's residential history. There are opportunities to study long-term AP exposures in relation to long-

term atherosclerotic processes using novel tomography-based measures such as coronary artery calcification (CAC). Another way to overcome this challenge in assigning temporality in the case of chronic exposure and cardiovascular disease is to focus the research on a vulnerable subpopulation where effects of the exposure can be measured in a shorter time-frame or where specific intermediate measures can link the exposure to the outcome. An important example may be to measure associations in middle-aged women, as a potential "at risk" and particularly informative population. In this population, age is a risk factor for both long-term exposure to AP and cardiovascular disease (CVD). Middle-aged women are of particular interest in this scenario given that the menopause transition is underway (93). The dynamic hormonal and metabolic changes in middle-aged women (159) may be important intermediaries between AP exposure and CVD risk. A focus on this subpopulation and these specific physiologic processes can improve measurement of the health effects of AP, give insight into biologic mechanisms, and support efforts to reduce chronic disease burden in women.

3.2.1 Background

Subclinical measures of atherosclerosis and particulate matter: potential pathways

Atherosclerosis, a progressive disease, is characterized by the plaque accumulation in the arteries (54). Along the biological pathway leading to a clinical coronary event, there are subclinical changes in the endothelium of the coronary and carotid arteries, which have been shown to be predictive of future cardiovascular risk (160). Coronary artery calcification (CAC) is a significant marker of SCA and coronary heart disease (CHD) (6,7). There is a direct correlation of coronary calcium measured by electron beam computerized tomography (EBCT) and atherosclerotic lesions in the arteries of the heart (161). Traditional cardiovascular risk factors,

including age, smoking, systolic blood pressure (SBP), diastolic blood pressure (DBP), high-density lipoprotein cholesterol (HDLc), and body mass index (BMI), are also significant predictors of CAC (162).

Controlled exposure and cellular experiments shed light on mechanisms linking $PM_{2.5}$ exposure with CAC. At the cellular level, cell types, including lung epithelial, endothelial cells, and cardiomyocytes, have shown to respond to in vitro PM exposure with elevated reactive oxygen species (ROS) levels and oxidative stress (96–98). With the controlled exposure, inhalation of the tiny particles (e.g. $PM_{2.5}$) deep into lungs and blood circulation through the alveolar-capillary membrane may stimulate an inflammatory response (e.g. increased levels of interleukin (IL)-6, nitric oxide (NO)) and decrease cell viability in human coronary artery epithelial cells, which impacts vascular health, further hardening arteries (100). The commonly proposed physiological pathways linking AP and CV events are through: 1) pulmonary oxidative stress or inflammation (this implies lung-specific inflammation); 2) endothelial dysfunction (blood vessel endothelium); and 3) atherothrombosis (100). The inflammation and coagulation systems' interaction is the key mechanism leading to endothelial dysfunction, which link to eventual CV events (100). Stimuli such as air pollutant exposure may initiate the development and progression of calcification through the death of smooth muscle cells (SMC), which could be the driving force for early microcalcification (56). This is then followed by infiltration of macrophages into the lipid pool, undergoing cell death and calcification, which leads to the development of a necrotic core processed along with inflammation (101). In addition, ROS regulate the activity of redox-sensitive signaling pathways and affect vascular biology (2). Inhalational exposure to AP triggers not only inflammation but also major ROS generated in AP exposure, which affects NO release, leading to endothelial dysfunction, with detrimental effect on vascular cells (2–5).

Diverse methods used in measuring particulate matter exposure

The commonly used methodology for $PM_{2.5}$ exposure measurements in epidemiology studies include, but is not limited to using AP levels from the nearest monitor data to the residence of individuals, estimating the city-wide average, utilizing an inverse-distance weighted estimate to a pollutant source, applying land-use regression or spatio-temporal models (25). The use of varying methodology of $PM_{2.5}$ exposure assessment across studies complicates our ability to make comparisons and establish generalizable knowledge of the association between $PM_{2.5}$ exposure and SCA. This is a focus of this review and our recommendations for future work in this area.

Is the menopause transition a critical window for air pollution and cardiovascular disease?

There may be age and gender differences placing certain subgroups at higher risk of AP adverse effects**.** Between 1999 and 2015, AP was associated with 16,000 deaths in US females, which was slightly more than men (~15,000) (1) and in the U.S. the number of CVD deaths for females has exceeded that for males. Inhalational exposure to $PM_{2.5}$ has also been linked to increased risk for women and the elderly (163), as women are at increased risk of coronary artery disease (CAD), overall mortality and cardio-metabolic disease after menopause (164). During the menopausal transition, a vulnerable period of accelerating CVD risk, women undergo adverse alterations of vascular remodeling, which are believed to be affected by more than chronologic aging alone (108,109). Therefore, midlife women could be a potential higher risk group for the effects of AP. The menopause transition is a dynamic physiologic process that could be a critical link between AP exposure and CVD risk. A previous study reported that women with premature or early menopause, defined by surgical intervention or premature ovarian failure, may also have an increased risk of non-fatal cardiovascular disease (165). The scientific assumption has been that

the subpopulation of early onset of menopause individuals or women undergoing the menopausal transition, (close to final menstrual period (FMP), one year after women's menopause) may be less protected from the harmful effect of AP relative to the risk of cardiovascular damage.

Approaches and challenges in estimation of the effect of $PM_{2.5}$ exposure on SCA in midlife women and proposed solutions

Along general pathways described above on how $PM_{2.5}$ exposure might contribute to adverse cardiovascular changes and increase CVD risk, there is evidence supporting a connection between $PM_{2.5}$ exposure and increases in inflammatory markers such as C-reactive protein (CRP), plasminogen activator inhibitor Type 1 (PAI-1) and homocysteine among midlife women (93,166). Among middle-aged women, there are alterations in estradiol (E2) level during their menopausal transition, which could be the reasons supporting the connections between $PM_{2.5}$ exposure and CVD risk during women's midlife. Interestingly, studies have shown links between the menopausal transition and adverse alterations in low-density lipoprotein cholesterol (LDL-C), and E2 levels among non-obese women (119,167), which could increase their CVD risk. Therefore, it is possible that these physiological changes during the menopausal transition, involving changes in E2 (168), and inflammation markers (169) (such as CRP), could augment the adverse effect of $PM_{2.5}$ exposure on SCA. Because those unique physiological changes described above, women and men need to be studied separately to estimate effects of AP on CAC. Application of a mediation analysis and effect decomposition that allow decomposing a total effect into a direct and an indirect effect (170) may shed light on how the independent variable ($PM_{2.5}$) influences the mediator variables (inflammatory markers, hormones, etc.), which in turn influences the dependent

variable (SCA, e.g. CAC). Thus, the intermediary variables may clarify the nature of the relationship between the independent and dependent variables, as described in **Figure 3.1**.

3.2.2 Objectives

This review will focus on the exposure measurement methodology and results of the studies of $PM_{2.5}$ and SCA, measured as CAC in the general population. Particular attention will be paid to describing theories of the underlying biology in the general population and in women undergoing the menopause transition. Through a synthesis of the literature, we will make recommendations for future studies in women in midlife. We hope to identify research gaps based on conducted review and propose a conceptualized model to better evaluate this association.

Studies published from 2005 to the present which considered the long-term impact of $PM_{2.5}$ and its constituents on SCA risk using a measure of CAC were included in the review (8–14). Another important measure of SCA, carotid Intima medial thickness (CIMT) has been also associated with $PM_{2.5}$ and other air pollutants. Although CIMT is not as predictive of incident CVD as CAC, midlife women experience significant increases in CIMT during the menopausal transition making this measure of specific interest. Therefore, we also reviewed additional studies (12,83,144,165) assessing the association between $PM_{2.5}$ exposure and CIMT of the carotid arteries, and differences in this association by gender or age.

3.3 Methods

Evaluate the association between long-term $PM_{2.5}$ exposure and CAC

To identify published research on the association between $PM_{2.5}$ exposure and CAC, we used the National Library of Medicine (PubMed) on March 11, 2020, including all investigations using atherosclerosis with AP as MeSH terms for our search. The keywords ("particulate matter"[MeSH Terms] OR "air pollution"[MeSH Terms]) were combined with (("atherosclerosis"[MeSH Terms]) OR ("atherosclerosis, coronary"[MeSH Terms]) OR ("coronary artery calcification"[MeSH Terms]) OR (“subclinical atherosclerosis”[MeSH Terms])), for the search. All titles were initially reviewed from the search to screen and the relevant and original studies addressing associations between AP and SCA among women from 2005-2020 were selected. Additionally, only English language studies, those involving humans and those MEDLINE journals were included. The epidemiologic investigations with cross sectional or longitudinal cohort designs were included. After a complete review of all abstracts, and full texts, there were seven separate publications assessing the long-term exposure to $PM_{2.5}$ and the risk of SCA as measured by CAC (**Figure 3.2**). Long-term $PM_{2.5}$ exposure was defined by our review as those assessing cumulative exposures and we included only those studies that evaluated the $PM_{2.5}$ long-term exposure and its chronic effect to CAC (not acute effect). There are several different ways the long-term exposure of $PM_{2.5}$ has been described within the context of these seven studies and those will be defined and evaluated.

For the selected studies on the associations of $PM_{2.5}$ exposure and CAC, information related to study design, population sources and sizes, and general demographic characteristics was extracted; differences in exposure assessment as well as method of outcome measurement, primary findings, adjustment factors, and specific effect modification terms were noted. Specifically, we

summarized findings to date and proposed models going forward that may improve the study of the association of $PM_{2.5}$ and CAC in women over the lifespan but particularly within midlife. We compared and contrasted each of these studies for the following components: 1) design of each investigation; 2) population at risk/clinical measures including age, gender and race/ethnicity; 3) exposure assessment methodology (e.g. how $PM_{2.5}$ was estimated); 4) timing of exposure measures and the definitions of long-term exposure; 5) specific results of the association of $PM_{2.5}$ and CAC, including risk estimates and 95% CI confidence intervals in general population, or men and women when applicable. Additionally, a descriptive summary and synthesis of associations between $PM_{2.5}$ exposure and CAC are provided, with a focus on whether age and gender differences were evaluated in those studies.

Evaluate the association between long-term $PM_{2.5}$ exposure and carotid Intima medial thickness (CIMT)

Studies that reported on associations of long-term $PM_{2.5}$ exposure and CIMT were also reviewed because changes in CIMT have been observed during the menopause transition. As our main interest and focus of this review is on the studies of $PM_{2.5}$ exposure and CAC, we did not conduct a systematic review for the studies assessing associations of $PM_{2.5}$ exposure and CIMT. Instead, we had a particular focus on those studies that evaluated gender or menopausal status as effect modifiers of $PM_{2.5}$ exposure and CIMT association. For those studies, we focused on summarizing the role of gender and menopausal status on $PM_{2.5}$ exposure and the CIMT association. This will help us better understand the physiological pathways among the potential "at risk" population of midlife women.

3.4 Results

3.4.1 Effects of $PM_{2.5}$ exposure on CAC among the general population as well as in women

3.4.1.1 Design of each investigation

Seven studies assessing the association between ambient AP ($PM_{2.5}$ exposure) and CAC were reviewed (**Figure 3.2**). These represent four primary cohorts. They include 1) four separate investigations from the Multi-Ethnic Study of Atherosclerosis (MESA) by Die Roux et al., 2008; Sun et al., 2013; Kim et al., 2014; Kaufman et al., 2016; 2) one study from the German Heinz Nixdorf Recall Study (HNRS) by Hoffmann et al., 2007; 3) one study from the Framingham Offspring cohort by Dorans et al., 2016; and 4) the last published piece of work, which was from the Coronary Atherosclerosis Disease Early Identification and Risk Stratification by Noninvasive Imaging (CREATION) Chinese cohort by Wang et al., 2019 (8–14). Among those identified four cohorts (US MESA, US Framingham offspring, German, and Chinese), except for the most recent MESA work in 2016 (10) and the study conducted using the Framingham Offspring cohort in 2016 by Dorans, all of the studies assessed the association using a cross-sectional study design (**Table 3.1**).

The four studies assessed the $PM_{2.5}$ and CAC association using the MESA cohort collected CAC data at baseline (2000-2002) (8–10,12), and the one longitudinal study also utilized CAC repeated measures data collected at follow-up visits from 2002 to 2005 and from 2010 to 2012 (10). In addition, the German HNRS study by Hoffmann et al., 2007 utilized baseline data (2000-2003) on 4,494 men and women from a highly industrialized rural area in Germany (11). The aim of the original HNRS study was to improve the prediction of cardiovascular events by integrating new imaging and non-imaging modalities in risk assessment (171), so the air pollution data was

added to this cohort later for evaluating the association with CAC. Furthermore, the Framingham Offspring cohort by Doran et al., 2016 included 3,399 participants and 51% of them were men. The inception of the Framingham Offspring cohort was investigating the familial clustering of CVD phenotypes and the role of shared environmental factors versus genetic factors in contributing to such aggregation (151). The air pollution data were added to this cohort by Dorans et al, 2016 and they conducted the study assessing the association between $PM_{2.5}$ and CAC presence and progression. The last published piece of work (Wang et al, 2019) came from the CREATION Chinese study (14). The data were collected from 8,168 outpatients of adults aged 25 to 92 with suspected CHD in 2015 to 2017 at baseline (14). The major original aim of this study was to identify risk factors that lead to coronary artery atherosclerotic plaque progression and events of CAD (14), and Wang et al, 2019 added the air pollution estimation data to conduct their study.

3.4.1.2 Population at risk/clinical measures

The characteristics of each reviewed study, summarized in **Table 3.1**, including age and gender distribution, as those are two key of the factors of interest. The US MESA, US Framingham Offspring, German HNRS, and Chinese CREATION studies had similar study hypotheses to determine the relationship between $PM_{2.5}$ exposure and CAC presence or progression (8–14). The four most common ethnic groups in the United States were included in MESA, and CAC was found to be higher in white patients (10). The HNR study group (mean age=60.2; range: 45-74) was slightly younger than the MESA cohort (mean age=62; range: 45-84 years) and included some individuals with previous CV disease (CVD) (172) . The Framingham Offspring cohort had an average age of 52.2 and 59 years old at baseline and at follow-up CAC measuring visits, and around half of them were women (13). The Chinese CREATION study included a wide age range

(age range: 25-92 years), with an average age at baseline of 56.9 (SD: 10.4) (14). Of all participants across reviewed studies, 46.4-53% were women, and the average age of the general population ranged from 52.2 to 62 years old (exception: the Chinese CREATION cohort had very wide age range: 25-92 years). When comparing the two longitudinal studies, the MESA cohort by Kaufman et al., 2016 was older, on average, than was the Framingham offspring cohort (62 vs. 52.2 years old), and it had more female participants (53% vs. 46.4% female) (**Table 3.1**).

3.4.1.3 Exposure assessment methodology

In this section, we summarized the exposure assessment measures in detail for each of the included studies. Overall, the studies we reviewed bring together varied assessment techniques in assigning exposure within the four cohorts. Early on in these studies, instead of a PM level, investigators used distance from a main road to the residence (11,13). More recently, the exposure has been estimated based on hierarchical models including nearest monitor, inverse-distance monitor weighting, and city-wide average (9,10,12,14).

Diez Roux, et al., 2008 (MESA) imputed mean $PM_{2.5}$ data, using a spatial-temporal model (12). In addition, they also estimated mean 2001 $PM_{2.5}$ exposure for each participant by averaging available daily data collected at the nearest monitor of the residential address at baseline (12). There was significant heterogeneity in the level of exposure between the six study sites (Baltimore, Maryland; Chicago, Illinois; Forsyth County(Winston-Salem), North Carolina; Los Angeles County, California; New York City, NEW York; and St. Paul, Minnesota), with PM varying from 12.82 (SD=0.71) in Minnesota to 24.10 (SD=3.29) $\mu g/m^3$ in California (12). In another MESA study conducted by Sun et al, 2013, they looked at the $PM_{2.5}$ components (sulfur, elemental carbon, silicon and organic carbon) when evaluating the association with CAC. Three approaches were used for exposure assessment: nearest monitor, inverse-distance monitor weighting, and city-wide

average (8). In the study conducted by Kim et al., 2014, two advanced exposure prediction modeling approaches were used, and those approaches have been described in detail elsewhere (173,174). Briefly, annual averages of predicted $PM_{2.5}$ components' concentrations at baseline participant addresses were estunated for one year from May 2007 to April 2008 when data on all four $PM_{2.5}$ components were available (9,174). Unfortunately, the modeling was based on 2009-2010 levels of the $PM_{2.5}$ as testing was not available during the study baseline period (2000-2002), which lead to exposure misclassification (9). This use of levels from misaligned years could introduce significant bias, as we know there has been a significant downward trend in AP for the past 20 years. The authors stated that the study intent was to obtain the most valid and precise measures of association possible, and therefore they predicted individual-level concentrations based on a rigorous exposure modelling approach (9), which is a more accurate approach compared to that used by Sun et al., 2013 (8). MESA (Kaufman et al, 2016) used average measurements from a cohort-focused monitoring campaign, the spatio-temporal model employed, incorporating community specific measurements, agency monitoring data, and geographical predictors, for $PM_{2.5}$ exposure estimation (10).

Hoffmann et al, 2007 (Germany HNRS) estimated exposure using the residence-based approach, and the daily mean value for $PM_{2.5}$ for 2002 was calculated for each grid cell. In addition, they also estimated distances between residences and major roads using official digitized maps and categorizing them into high traffic exposure ($\leq$100 m) vs. low traffic exposure categories (11). Dorans et al., 2016 in the Framingham Offspring study relied on residential distance from a major roadway as well as residential $PM_{2.5}$ exposure. Spatially resolved average $PM_{2.5}$ exposures were used for exposure assessment (13). Averaged daily total $PM_{2.5}$ predictions over the same index year were used to estimate the annual average $PM_{2.5}$ exposure for all participants (13). Wang et al.,

2019 used hierarchical land-use regression modeling for $PM_{2.5}$ exposure assessment on the basis of annual mean daily monitoring data (2014-2015) from the nationwide monitors (14). In addition, this study further tested different $PM_{2.5}$ exposure time window impacts on CAC, including cumulative exposures going back for 3-6 years and for 10-year periods before baseline CAC measurement (14).

The mean level of $PM_{2.5}$ exposure reported by the US MESA studies (by Diez Roux et al. in 2008; Kim et al. in 2014; Sun et al. in 2013; and Kaufman et al. in 2016) and German HRNS study (by Hoffman et al, in 2007) ranged from 13.7 to 22.8 $\mu g/m^3$ (8–12). The median value in the US Framingham Offspring Study was 10.7 $\mu g/m^3$; and the average was 70.1 $\mu g/m^3$ (SD=20.0) in the Chinese study (13,14).

In summary, several different methods for exposure assessments were used for air pollutants exposure estimation in the studies evaluating the association between $PM_{2.5}$ and CAC. The use of different methodologies of exposure assessment across studies may have led to the very different findings reported. Overall, those studies that employed spatiotemporal exposure assessment are less prone to exposure misclassification than the studies that used monitor-based measures, thus, given the better quality exposure assessment.

3.4.1.4 Timing of exposure metric and measures of $PM_{2.5}$ long-term exposure

Hoffman et al in the HNRS study used a residence-based approach to estimate $PM_{2.5}$ with the European Air Pollution Dispersion model for an annual average for 2002, and the study period was 2000-2003 (11). They also added a proximity to major roadways estimate as a surrogate for $PM_{2.5}$ (11).

The longest measure of long-term exposure was carried out by Diez Roux (MESA) who estimated a 20-year exposure of $PM_{2.5}$ for 1982-2002 by using geocoded residence over time and

the closest monitor for all addresses within 13.1 km of a monitor (12). Sun et al, 2013 MESA study built upon the Diez Roux model with organic carbon (OC) and elemental carbon (EC) components of $PM_{2.5}$ and using community monitors for 2007 to 2009 (8). Community monitors deployed for the MESA study started from 2005 and ended in 2008, and the Sun et al., 2013 study used 2007-2008 monitors' estimate exposures for baseline measures (2000-2002), as the monitored data only became available several years after baseline (8). The endpoint measures were obtained during the period 2000-2002 (8). Exposures were likely misclassified because the exposure measures were collected 5 to 8 years later than the endpoint outcome measures. The authors addressed the exposure misclassification by assigning "future" exposure to the participants in their final analyses. They justified this by stating that there was a good correlation over the 6-year MESA study period (2002-2007) (8). However, their exposure assessment of assigning a later-than-outcome assessment time exposure to the participants (extrapolating exposures) was not accurate, compared with assigning the exposure prior to the outcomes were measured. Kim et al, 2014 (MESA) looked specifically at only chemical components of $PM_{2.5}$ using 2007-2008 modeled spatio-temporal estimates (9). Finally, Kaufman et al, 2016, as a final MESA project, was the only study to consider progression of CAC, with a large sample size and an average of 6.2 years' follow-up. Participant-specific $PM_{2.5}$ exposure averaged over the years 2000-2010 ranged from 9.2-22.6 $\mu g/m^3$ (10). $PM_{2.5}$ exposure estimations were from 1999 to 2012, while the CAC measures were done at baseline (2000-2002) and at follow-up visits (2002-2005; 2005-2007; 2010-2012) (10).

The Framingham cohort study (Dorans et al, 2016) adopted a 2003 average $PM_{2.5}$ from a spatio-temporal model as well as an average for the entire period of 2003-2009. They also included a measure of residential proximity to a major roadway, which is a surrogate for exposure to local traffic emissions (13).

Wang's study in 2019 of 8,867 individuals in China followed people from 2015-2017, but used 2014-2015 annual mean daily monitoring data for estimating long-term air pollution concentrations for each participant (14). The annual mean levels of $PM_{2.5}$ exposures were 70.1 (SD=20.0) $\mu g/m^3$ (14).

In summary, different timing of exposure metric and measures of $PM_{2.5}$ long-term exposure were used for air pollutants exposure estimation in the studies evaluating the association between $PM_{2.5}$ and CAC. Overall, those studies that used one to two years prior to outcome's air pollution are less likely to have exposure misclassification compared to the other studies included in this review. Average annual mean levels of $PM_{2.5}$ from one or a few years before the outcome measure provide better quality of the exposure assessment of long-term $PM_{2.5}$.

3.4.1.5 Results of the association of $PM_{2.5}$ and CAC, including risk estimates and 95% confidence intervals

Studies that reported null or equivocal findings

Five of the seven investigations have yielded equivocal or null findings related to long-term $PM_{2.5}$ exposure and subsequent CAC measurements (8,9,11–13). All of the adjustment covariates in each reviewed studies for the risk estimates were included in the **Table 3.2**. In the German HNRS cohort, Hoffman et al, 2007 reported that $PM_{2.5}$ exposure was not associated with higher CAC in all subjects (12.7% higher CAC per IQR increase in $PM_{2.5}$ <3.91 $\mu m/m^3$>, 95% CI: -5.6, 45.5) (11). However, those who lived 50m from a major road had a statistically significant higher chance for a high CAC (OR=1.63, 95% CI: 1.14-2.33) compared to those who lived living >200 m away from a major road; and a strong association in men and younger participants was reported (11). In subgroup analyses, the study reported that $PM_{2.5}$ exposure showed stronger effects

on CAC among the participants who had not been working full-time in last 5 years before the baseline visit (11). Within the MESA study, Diez Roux et al, 2008 failed to find a statistically significant association between $PM_{2.5}$ exposure and CAC. In addition, they found no evidence that long-term $PM_{2.5}$ exposure was more strongly associated with CAC in subgroups, including women and older people (12). Later analysis within the MESA cohort by Sun et al, 2013 showed that none of the evaluated $PM_{2.5}$ components was associated with risk of CAC. No effect modifications were evaluated in this study (8). In other work from MESA published in 2014, Kim et al utilized a different modelling approach than Sun et al, 2013 discussed above. Regardless, they were not able to detect an association between CAC and any of the modeled $PM_{2.5}$ exposures (9). In addition, the study did not evaluate any effect modifiers, neither by gender nor by age. Finally, using the Framingham Offspring Cohort participants, Dorans et al (2016) did not observe any association between $PM_{2.5}$ exposure and any CAC in the region with relatively low levels of and little variation in $PM_{2.5}$ exposure (median $PM_{2.5}$ in 2003=10.7 $\mu g/m^3$ and range: 2.9-26.7 $\mu g/m^3$) (13). Interestingly, their subgroup analyses, which were published in supplemental material, showed a stronger association between $PM_{2.5}$ and CAC among men than women, though the interactions evaluated were not statistically significant (OR for male: 1.03, 95% CI: 0.92, 1.15 vs. female: 0.92, 95% CI: 0.82, 1.04) (13).

Studies that are supportive of an association between $PM_{2.5}$ exposure and CAC

Kaufman et al. in 2016 assessed associations of progression of SCA with ongoing exposure of $PM_{2.5}$ (10). This longitudinal cohort work of MESA showed a statistically significant positive association between $PM_{2.5}$ exposure and CAC progression which considered CAC measurements over time (Agatston unit yearly change per IQR $PM_{2.5}$ exposure (5 $\mu m/m^3$) increase: 4.1 (95% CI: 1.4 to 6.8)) (10). They also reported that the associations between $PM_{2.5}$ and CAC progression

were stronger among women and those older than 65 (specific data on the risk estimates were not available) (10).

Wang et al (2019) utilized a cohort of Chinese participants and found a positive association between long-term $PM_{2.5}$ exposure and CAC severity (Agatston unit % increase per $PM_{2.5}$ exposure IQR increase (30 $\mu m/m^3$): 27.2 (95% CI: 10.8 to 46.1)) (14). They concluded that $PM_{2.5}$ exposures were independently positively associated with CAC. In addition, they evaluated interactions of the exposure response by age and gender, and found that $PM_{2.5}$ exposure effects on CAC were stronger among males (percent change in CAC (95% CI): 42.2 (24.3, 62.7) vs. females: 17.6 (2.6, 34.8)) and among those older than 60.

In summary, as described earlier, both exposure ($PM_{2.5}$) and outcome (CAC) have issues with measurement, including temporality and spatial issues and biased assessments. The investigators tried to deal with confounding in their different populations. The majority of the studies adjusted for age, gender, race, socioeconomic factors (e.g. education, income, area of residence), BMI, physical activity, smoking, and some studies further adjusted for lipid levels (e.g. HDL, LDL, triglycerides), blood pressure, physical activity, alcohols consumption, diabetes, hypertension, and lipid-lowering medication, when assessing the association between long-term $PM_{2.5}$ and CAC (Table 3.2). Results need to be interpreted with caution because adjustment for those factors (e.g. potential mediators) for assessing the association is not optimal for mediation analyses as doing so may block the pathway. These previous studies adjusted for covariates to explore whether those covariates (risk factors) alter the association between $PM_{2.5}$ and CAC. To summarize the reviewed risk estimates, non-statistically significant findings were reported by investigators in all cross-sectional studies related to $PM_{2.5}$ exposure and CAC presence association (8,9,11,12), except for one Chinese study by Wang et al., 2019 (14). In addition, the longitudinal

design was adopted for two of the investigations of $PM_{2.5}$ exposure on CAC progression. Kaufman et al., 2016 US modelled CAC progression and its association with $PM_{2.5}$ exposure and reported a positive association (10). However, the Framingham Offspring cohort study by Dorans et al., 2016 evaluated the association between $PM_{2.5}$ exposure and CAC presence and progression, and found no significant relationship (13).

3.4.2 Effects of $PM_{2.5}$ exposure on CAC among women

Four out of the 7 studies examined effect modification by sex or age with $PM_{2.5}$ to CAC risk (10,11,13,14). Although all four studies reported that the assessed interactions were not statistically significant, two of the studies concluded that the effect of $PM_{2.5}$ exposure to CAC was stronger in subgroup analyses of the elderly and women (10,14) (**Table 3.2**). Kaufman et al., 2016 (MESA) study found that the association between $PM_{2.5}$ and CAC progression was stronger among women, though the evaluated interactions were not statistically significant (10). The study by Wang et al., 2019, conducted further sensitivity analyses among postmenopausal women (N=1732 out of 3790 women, 45.7% postmenopausal women), and reported that the positive association of $PM_{2.5}$ exposure and CAC was significant and much stronger among this subgroup of women (34.5% greater CAC score for a 10 $\mu g/m^3$ increase in $PM_{2.5}$; 95% CI: 5.8% to 70.9%) (14). These findings indicate that there is a potential stronger effect among women or among women after menopause for $PM_{2.5}$ exposure to CAC development.

The remaining six studies assessed association between $PM_{2.5}$ exposure and CAC neither adjusted for menopause status nor were able to assess the menopausal transition as an effect modifier for evaluating the association between $PM_{2.5}$ exposure and CAC (8–13).

3.4.3 $PM_{2.5}$ and CIMT: effect of gender or age as modifier

We have also reviewed the studies assessing the $PM_{2.5}$ exposure and CIMT risk association with a focus on looking at those studies, which particularly assessed gender or age as effect modifier. We found five studies, which examined effect modifications by gender (12,83,144). **Table 3.3** includes information on study design, cohort, and evaluated effect modification in detail. Overall, findings suggest that there is no statistically significant interactions of gender or age on the $PM_{2.5}$ exposure and CIMT association.

One cross-sectional study (N=798), by Kunzli et al., 2005, used two clinical trials' data from the Vitamin E Atherosclerosis Progression Study (VEAPS), and the B-Vitamin Atherosclerosis Intervention Trial (BVAIT). The study exposure was annual mean $PM_{2.5}$ (ranged: 5.2-26.9 $\mu g/m^3$; mean=20.3), assigned to participants using their geocoded residential areas. This study reported that the association between $PM_{2.5}$ exposure and CIMT was strong among older people (>= 60 years), and women, with the strongest association found in women >= 60 years (N=186 out of 355 women) (% difference in CIMT=15.7%, 95% CI: 5.7-26.6%) (83). However, they reported that there was no clear evidence of associations of $PM_{2.5}$ exposure with CIMT by age or sex, as all their evaluated p for interactions were greater than 0.05 (83).

The study by Diez Roux et al (2008), which used the MESA cohort (N=5172), also evaluated the association between $PM_{2.5}$ exposure to CIMT, along with CAC as summarized above. The exposure data were obtained from a space-time model of EPA monitor data linked to participants' residential history data. A weak positive association was reported for $PM_{2.5}$ exposure and CIMT, controlling for age, sex, race/ethnicity, socioeconomic factors, diet, smoking, physical activity, blood lipids, diabetes, hypertension, and BMI (1-4% increase per 12.5 $\mu g/m^3$ increase in

$PM_{2.5}$) (12). The heterogeneity of effects was examined by this study, and they reported that there was no evidence of any assessed effect modification, including age and sex (12).

Kunzli et al., 2010, used data from five clinical trials (the VEAPS, the BVAIT, the Estrogen in the Prevention of Atherosclerosis Trial, the Troglitazone Atherosclerosis Regression Trial, and the Women's Estrogen Lipid-Lowering Hormone Atherosclerosis Regression Trial) to conduct a longitudinal analyses for assessing the association between $PM_{2.5}$ exposure and CIMT progression (147). $PM_{2.5}$ were derived from a geo-statistical model with data from the monitoring station and home outdoor mean concentrations were estimated (147). A positive, but not statistically significant association was reported for $PM_{2.5}$ exposure and CIMT progression. For the effect modifications they tested, they found that associations did not differ by sex (147).

Another cross-sectional study conducted by Bauer et al, 2010 using the HNRS cohort estimated residential long-term exposure to $PM_{2.5}$ using a chemistry transport model (145). The interdecile range increase in $PM_{2.5}$ was 4.2 $\mu g/m^3$, and was associated with a 4.3% increase in CIMT (145). They evaluated several effect modifiers, including age and sex and found no statistically significant differences in the associations between $PM_{2.5}$ exposure and CIMT (145).

Furthermore, Adar, et al., 2013, using the MESA cohort data evaluated the cross-sectional and longitudinal associations between $PM_{2.5}$ exposure and CIMT (144). The $PM_{2.5}$ exposure was estimated over the previous year's baseline and between ultrasounds of CIMT using a spatio-temporal model. This study suggested that higher long-term $PM_{2.5}$ exposures were associated with increased CIMT progression and the reductions in $PM_{2.5}$ exposure were related to slower CIMT progression (144). Among their evaluated effect modifiers, a stronger association was detected for women, but not for age (144), although this tested interaction was not statistically significant.

In summary, our review found that some studies indicate that women were at higher risk of $PM_{2.5}$ exposure effects on CIMT as compared to men (83,144). However, although most of the above studies' evaluated effect modifications by age or gender, and some reported higher risk among women, those findings of their evaluated effect modifiers were all not statistically significant. Therefore, we conclude that our reviewed studies found no interactions by either gender or age between the $PM_{2.5}$ exposure and CIMT association.

3.5 Discussion

Our review found that among the general population there is equivocal evidence with some, but not all studies reporting positive associations between $PM_{2.5}$ long-term exposure and CAC (8–12,14). The seven reviewed studies were identified between 2005 and 2020 and included data analyses using varying exposure metrics representing four cohorts that had either prospective or retrospective-prospective data and could then be equated with long-term exposures. Those cohorts are: the U.S. Multi-Ethnic Study of Atherosclerosis (MESA), the German HNRS, and the U.S. Framingham Offspring cohort study (8–13), and an additional international cohort study was recently conducted in China in 2019 (14). All the identified cross-sectional studies, except one, reported no evidence of an association between $PM_{2.5}$ exposure and CAC presence. The only exception is the Chinese cohort study, which showed a positive association (14). In longitudinal study settings, when considering CAC progression over time and its association with $PM_{2.5}$ long-term exposure, there was evidence of a positive association (10). In support of the proposed hypothesis, in a subgroup of postmenopausal women, the associations between $PM_{2.5}$ exposure and

CAC were reported to be even stronger, compared with premenopausal women of the Chinese cohort (14).

Our study identified four additional investigations on this topic for reviewing and reported a more in-depth approach focusing on women than a previous conducted meta-analysis (described below) on the similar topic. Additionally, based on our thorough review, we were able to identify the group potentially sensitive to air pollution group in relation to CAC risk, postmenopausal women or elderly, which brought to light many questions in need of further investigation. The meta-analyses conducted previously by Akintoye et al., 2016 assessed the association between $PM_{2.5}$ and SCA, although only three studies evaluated this association were included in this meta-analyses (149). As two out of their included three studies used the same cohort, the U.S. MESA study cohort (8,12), they had small numbers in the included studies. The authors therefore concluded that they have been underpowered to find a meaningful association as they found a low number of studies that evaluated the association between $PM_{2.5}$ and CAC (149).

The biological mechanisms for the cardiovascular effects of PM exposure have been proposed by previous studies, but greater understanding of how $PM_{2.5}$ long-term exposure affects CAC is needed in order to prevent and minimize the deleterious health effect. According to a recent published finding, exposure to $PM_{2.5}$ is hazardous to health and was associated with over 30,000 deaths from cardiorespiratory diseases in 2015 in the U.S (1). In the most recent AHA statement, a small positive, yet consistent association between PM and CVD events was reported (22).In addition, several observational studies have reported a positive association between fine particulate AP and increased risk for cardiovascular mortality and atherosclerosis (10,83,84). Although the biological mechanisms linking the association between $PM_{2.5}$ exposure and CAC among human studies are not fully confirmed yet, animal studies provide some firm evidence on mechanisms

linking $PM_{2.5}$ exposure with CAC. Studies in mice and rabbits reported that inhalation of PM produces an inflammatory response and oxidative stress, linking air pollutants to atherogenesis (99,124,175). An animal model demonstrated that long-term exposure to ambient $PM_{2.5}$ leads to endothelial dysfunction and accelerates the progression of atherosclerosis, especially among mice deficient in apolipoprotein E or low-density lipoprotein receptor, which develop advanced atherosclerotic lesions (99). $PM_{2.5}$ exposure may also stimulate an inflammatory response and cytokine release, leading to altering vasomotor tone and lipid peroxidation (176). In addition, other potential mechanisms linking $PM_{2.5}$ exposure and progression of atherosclerosis include the effect of AP on blood pressure, autonomic function, and low-density lipoprotein oxidation (177–179). Considering those potential links, when evaluating the association between $PM_{2.5}$ exposure and SCA, reasonable confounders need to be considered carefully for modelling adjustment and exploring the pathways of the associations based on the available physiological mechanisms. Those potential biological mechanisms summarized above are for all populations, and not specific to menopause or female sex.

A limitation of the existing work that evaluated the association between $PM_{2.5}$ exposure and CAC risk is that most of the studies included only looked at the overall association in the general population. While only one recent study, by Wang et al, 2019, evaluated this association among the subgroup of postmenopausal women, and reported a stronger association than the rest of the women who participated in the study (14), no study has evaluated the effect of $PM_{2.5}$ exposure on CAC progression in women to understand why the effect is stronger among the postmenopausal. This stronger effect that was reported by Wang et al, 2019 was consistent with previous observational studies reporting that CVD risk increases after menopause and an American Heart Association statement listing menopause as a female-specific CVD risk factor (44). This is

an important gap because previous studies suggest that women are particularly vulnerable to SCA risk as they transition through menopause (180,181). Thus, the impact of $PM_{2.5}$ exposure on SCA could be heightened during this vulnerable menopausal period. The MESA cohort, which most of the studies (4 out of the 7 studies we reviewed) that evaluated $PM_{2.5}$ exposure and CAC association used, included an older population (mean age=62), in which most of the women were postmenopausal; therefore, they were not able to assess the impact of the menopausal transition on $PM_{2.5}$ exposure and CAC risk. A study with a younger female population, including those who are before and going through the menopause, will be needed to assess the $PM_{2.5}$ exposure and CAC association.

Evidence supports that midlife women could be the more vulnerable population to the effects of $PM_{2.5}$ exposure than general populations for SCA. Two of the reviewed studies above on $PM_{2.5}$ exposure and SCA by CIMT indicated the potential stronger effects among women than men (83,144). In addition, among those studies that assessed $PM_{2.5}$ exposure and SCA by CAC, the MESA study by Kaufman et al., 2016 and the Chinese study by Wang et al., 2019 both reported a stronger effect of $PM_{2.5}$ to CAC among women than among men, and a stronger effect on postmenopausal women than on premenopausal women. That evidence indicates that the biological changes in midlife women need to be further studied over time, to discover what makes them vulnerable.

Previous studies on the biological changes occurring among women, which may affect their cardiovascular health during their midlife, may provide support for what to look at to fill the next research gaps. A study using both the Healthy Women Study cohort and the Women's Healthy Lifestyle Project cohort reported that hormone-regulated changes, which commonly occur during menopausal transition, could potentially increase CVD risk and progression of subclinical disease

(102). In addition, SWAN and a few other studies have also addressed this vulnerable period of menopausal transition for women effected by their reproductive or biological changes (72,88). Furthermore, in the Framingham Heart study and Nurses' Health Study, natural menopause was found to be associated with increased CHD risk (though this study did not adjust for age and smoking) (182). Another study conducted in 2018 by JO et al. reported that the menopausal transition is associated with increased carotid arterial stiffness, independent of age (183). Furthermore, a Framingham Heart study described that the impact of menopause is crucial and abrupt, and may augment afterwards over time but that augmentation would happen slowly (184). Due to the biological changes occurring during the menopausal transition, the timing of CAC measures closer to FMP, where changes that are more obvious occur (e.g. E2) may augment the impact of $PM_{2.5}$ exposure to the CAC progression. Those changes during menopause could potentially become proatherogenic and modify the association between $PM_{2.5}$ exposure and CAC. The above findings provide insights that the changes between CAC got measured and the FMP could be crucial to study for exploring the role of menopause transition in the $PM_{2.5}$ exposure and CAC association. Using the time since FMP as a time scale to conduct a longitudinal study for assessing the association between $PM_{2.5}$ exposure and CAC progression over time could be one way to understand the role of menopausal transition on the association. In summary, studying women undergoing the menopause transition is crucial to determine if the menopause transition contributes to the stronger association between $PM_{2.5}$ exposure and CAC risk.

To disentangle the effects of menopause transition and chronological aging on the $PM_{2.5}$ exposure and CAC risk association, a longitudinal study with measures of the physiological changes during menopausal transition might be helpful to identify independent effects. There is an important opportunity going forward to incorporate women in midlife and the effects of chronic

air pollution exposure on biomarkers of inflammation, hormonal measures and their commensurate effect on cardiovascular disease. Future investigations both in the US and abroad are needed. However, the data with regard to the role of menopausal transition when assessing the association between $PM_{2.5}$ exposure and CAC is not well substantiated, as this information was not collected within each investigation over time. As summarized above, the only work assessing menopausal status's effect on $PM_{2.5}$ exposure and CAC presence association looked at baseline menopausal status cross-sectionally only (14), as they did not have further data available to explore the potential physiological changes occurring before or around menopause. This limited their ability to evaluate further on how menopausal status change may affect the association between $PM_{2.5}$ exposure and CAC. The cross-sectional approach cannot take into consideration the heterogeneity of the changes occurring in women transitioning through menopause (e.g. changes in E2). Longitudinal analyses will be needed to explore the potential effect modification (e.g. factors changing over the menopausal transition) and their roles affecting the association between $PM_{2.5}$ exposure and development of CAC. Measuring and modeling the association with repeated measures of covariates' variables in a longitudinal framework will produce different insights, as compared to cross-sectional approaches.

To explore the pathways linking the association between $PM_{2.5}$ exposure and CAC during menopausal transition, it will be helpful to look at the relevant studies that investigated the associations between $PM_{2.5}$ and the potential mediators (e.g. CRP, E2), and between those mediators and CAC among middle-aged women. Moreover, we should also caution to differentiate the potential confounders and mediators when assessing the association between $PM_{2.5}$ exposure and CAC. Specifically, when estimating the association of $PM_{2.5}$ exposure and CAC, the true confounders would only be those related to participants' physical environment and those that led

them to live where they live (e.g. neighborhood), and indicators of social advantage (e.g. income, education, and race). However, the potential mediators could be those intermediaries in the pathway of the association between $PM_{2.5}$ exposure and CAC during menopausal transition, such as the markers of inflammation and reproductive health, as these can be affected by $PM_{2.5}$ exposure and further lead to increased risk of CAC. Furthermore, some of those intermediaries also fluctuate during menopause transition, making them possible time-varying effect modifiers or mediators of the $PM_{2.5}$ exposure and CAC association. To better understand what confounders, mediators, and modifiers a study should adjust for/evaluate when assessing the association between $PM_{2.5}$ exposure and CAC and to provide evidence for the proposed conceptual model, we discuss the key relevant findings of the previous studies below to come up with the proposed conceptualized model shown in **Figure 3.1**. As there are plenty of changes occurring during the menopause transition (e.g. E2, CRP) and many factors are affected by $PM_{2.5}$ exposure (e.g. CRP, cholesterol), this conceptual model is crucial to construct prior to evaluating the association between $PM_{2.5}$ exposure and CAC, especially among a population of middle-aged women transitioning through menopause. Based on the studies we reviewed that explored the potential links between $PM_{2.5}$ exposure and pathway variables, and between pathway variables and CAC among middle-aged women, we propose to construct the conceptual models as shown in **Figure 3.1** for future studies. Two potential pathways are proposed in the conceptual model: 1) $PM_{2.5}$ exposure affects inflammation markers (e.g. CRP) and that affects CAC; and 2) $PM_{2.5}$ exposure affects hormone levels (e.g. E2), which fluctuate during the menopause transition, and that affects CAC. We discuss each of those pathways further, providing supporting evidence summarized from previous literature below:

1. $PM_{2.5}$ exposure - inflammation markers - CAC, among middle-aged women

Using 1,923 women (age range: 42-54) from the study of women's health across the nation (SWAN), a multi-center, multi-racial/ethnic, longitudinal study [36], investigators reported that chronic long-term $PM_{2.5}$ exposure is associated with increased CRP levels (185). In another study by Ostro et al, 2014, the association between $PM_{2.5}$ and CRP was observed with an even stronger effect in several susceptible subgroups, including those with high blood pressure and older diabetics (86). In addition, another SWAN study, by Green et al, 2016, provided evidence that the exposures to $PM_{2.5}$ in the preceding year are associated with adverse effects on inflammation (e.g. CRP) and hemostasis (a physiologic response to vascular injury) for CV outcomes (88). What's more, a previous SWAN study by Wu et al, 2017 reported gaseous air pollutants (CO, NO_2, SO_2) and the associations with increased thrombotic potential (formation of a blood clot) and cholesterol metabolism disruption (89), which has been linked to atherosclerosis (186). Those studies provided insight on the potential pathway we could explore further through inflammation markers linking the $PM_{2.5}$ exposure and CAC association.

2. $PM_{2.5}$ exposure - hormone - CAC, among middle-aged women

Furthermore, there is evidence showing the linkage between $PM_{2.5}$ exposure and SCA through hormone level changes. Though the biologic mechanism of the interaction between $PM_{2.5}$ exposure and menopausal transition to the CAC development remains uncertain, experimental and animal studies have examined some potential mechanistic links. As indicated by the Nurses' Health Study, it is likely that decreasing estrogen over the menopausal transition may alter the lipoprotein profiles, which could contribute to the development of SCA (187). Another SWAN's study, by El Khoudary et al, 2016, reported that high E2 early decline group women (women with higher E2 levels before FMP and then lower E2 thereafter) were associated with lower odds of

plaque, compared to women with the low E2 trajectory (188). Based on these study findings, it is possible that during the menopausal transition, fluctuating estrogen is a potential explanation for causing women during this period to become more vulnerable to the effects of $PM_{2.5}$ exposure than general populations for SCA (189). In addition to the study finding by Wang et al, 2019 that there is a stronger positive association between $PM_{2.5}$ exposure and CAC risk, the above summarized findings also provide insight on the potential pathway linking the $PM_{2.5}$ exposure and atherosclerosis association through the changes during menopausal transition. Therefore, it is interesting to explore the role of menopause transition or hormone changes during the menopausal transition on the association between $PM_{2.5}$ exposure and SCA.

The evidence above shows that a longitudinal observational cohort with those variables available will be needed to apply this conceptual model for further adjusting and testing. Evaluating the role of menopausal transition on $PM_{2.5}$ exposure and CAC association using this conceptual model could help characterize the populations of individuals at high risk of CAC or high-risk period of increasing CAC risk.

3.6 Conclusion

In conclusion, our review found from previous observational studies that there is equivocal evidence with some, but not all studies reporting associations between $PM_{2.5}$ exposure and CAC presence or progression among the general population (8–14). In studies published to date, subgroups of postmenopausal and older women showed stronger associations between $PM_{2.5}$ exposure and CAC. We conclude that there was limited evidence for the association of $PM_{2.5}$ exposure in women and CAC, and little to no knowledge of the role of the menopausal transition

in this association. To date, no study has been carried out on middle-aged women who are undergoing the menopause transition to specifically study this association. One important reason to pursue this study design is to elucidate potential mediators and/or effect modifiers that are expressed as part of the menopause transition, which might improve estimation of AP effects on women's health. Key components of future research on this important question include longitudinal measures of changes (i.e. CAC progression) during menopausal transition. This will advance the field by narrowing the list of candidate pathways illustrated here, and increase knowledge of the unique effects of AP on women's health.

3.7 Tables and Figures

Table 3.1 Description of studies included for synthesis

Author, year	Study Design	Study population (Location, characteristics)	Pop size/#	Exposure Measurement	Outcome	Age, years Mean (range)	% female
Hoffmann 2007	Cross-sectional	Germany, 2000-2003 Heinz Nixdorf Recall Study (HNRS)	4494	Residence-based approach to characterize exposure; Daily mean value for $PM_{2.5}$ exposure for 2002 was calculated for each grid cell	CAC -Ln (CAC+1)	60 (7.8)	51
Diez Roux 2008	Cross-sectional	USA, 2000-2002 MESA	5172 for imputed PM; 5041 for 2001 mean PM	20-year imputed mean; and 2001 mean PM were estimated. Imputed exposure used spatio-temporal model; and mean 2001 $PM_{2.5}$ exposure was estimated for each participant by averaging all available daily values collected at the monitor nearest their residential address at baseline.	CAC 1) Presence of CAC: Agatston scores >0; 2) Ln (CAC) for persons with nonzero CAC	62 (45-84)	53
Sun 2013	Cross-sectional	USA, 2000-2002 MESA	6256	$PM_{2.5}$ exposure: 1) Nearest monitor (primary approach); 2) inverse-distance monitor weighting; 3) city-wide average	CAC 1) Presence of CAC: Agatston scores >0; 2) Ln (CAC) for persons with nonzero CAC	62 (45-84)	52
Kim SY 2014	Cross-sectional	USA, 2000-2002 MESA	5488; -2683, presence of CAC; -2805, no CAC	Long-term concentrations of $PM_{2.5}$ components at participants' homes were predicted using both city-specific spatio-temporal models and a national spatial model	CAC 1) Presence of CAC: Agatston scores >0;	61.9 (45-84)	52.3

Table 3.1 Continued

					2) Ln (CAC) for persons with nonzero CAC		
Dorans 2016	Longitudinal cohort	USA, 2002-2011 Framingham Offspring cohort study	3399 CAC round 1 measured in 2002-2005; CAC round 2 measured in 2008-2011, for 51% of participants	Spatio-temporal model $PM_{2.5}$ exposure and residential proximity of major roads	CAC 1) Detectable CAC: Agatston scores >0; 2) Ln (CAC) for persons with nonzero CAC; 3) Detectable progression of CAC between the 1st and 2nd round of scans; 4) Annual change in CAC per year	52.2 at 1st scan (SD: 11.7); 59 at 2nd scan (SD: 11.8)	46.4
Kaufmann 2016	Longitudinal cohort	USA, 2002-2012 (mean follow-up 6.2 yrs) MESA	6795 -5834 had at least two CAC scans; -961 had only baseline CAC scan	Individual-weighted $PM_{2.5}$ -integration of all exposure monitoring and modeling outputs into a final, individual-level prediction, for each 2-week period of follow-up, permitting time-varying time-location information	CAC 1) CAC mean progression rate over follow-up; 2) Ln (CAC+25)	62 (45-84)	53
Wang 2019	Cross-sectional	China, 2015-2017 CREATION	8168	Hierarchical land-use regression modeling, for $PM_{2.5}$ exposure, based on annual mean daily monitoring data; sensitivity analysis, estimated cumulative exposures back for 3, 4, 5, 6, and 10-year periods before 2015	CAC 1) Detectable CAC: CAC>0; 2) Severe CAC: CAC>400	56.9 (25-92; SD=10.4)	51

Pop size/#, population size; $PM_{2.5}$, particulate matter ≤2.5 μm in diameter

Table 3.2 Association between $PM_{2.5}$ exposure and the development of CAC

Author, year	Exposure	Outcome Assessment / Validation	Effect Measure (OR, RR, %change)	Risk Estimate, LCL to UCL (Lower 95% CI)	Adjustment Factors	Effect modification
Hoffmann 2007	$PM_{2.5}$ exposure	CAC Use of chest CTs for each participant. CAC score calculated by the Agatston score. Final CAC score was summation of CAC scores of all foci in the epicardial coronary system.	% increase per IQR increase (3.91 $\mu g/m^3$)	Positive associated, but NS: 17.2% (95% CI: -5.6 to 45.5)	Age, sex, city of residence, area of residence, education, smoking, physical inactivity, waist to hip ratio, diabetes, blood pressure and lipids	Subgroup analyses among participants who had not been working full-time during the last 5 years No effect modification by age or sex was found
Diez Roux 2008	$PM_{2.5}$ exposure	CAC Two chest CT per participant, calculated mean Agatston score; 50% had CAC>0	Relative prevalence	NS	Age, Sex, race, socioeconomic factors, BMI, hypertension, HDL-C, LDL-C, smoking, diabetes, diet and physical activities. The following variables were explored for effect modification: age, sex, lipid levels, site, education, race/ethnicity, diabetes, BMI, smoking	The following variables were explored for effect modification: age, sex, lipid levels, site, education, race/ethnicity, diabetes, BMI, smoking No effect modification by age or sex was found
Sun 2013	$PM_{2.5}$ exposure	CAC 49% had CAC>0, and their median CAC score are 86 (IQR: 270.5); Two chest CT per participant, calculated mean Agatston score	RR per IQR increase -Presence of CAC -ln (detectable CAC)	NS	Age, gender, race-ethnicity; total cholesterol, HDL cholesterol, smoking status, hypertension, lipid-lowering medication; education, income, waist circumference, body surface area, BMI, BMI2, diabetes, LDL, triglycerides; metropolitan area	Effect modification by variables not assessed

Table 3.2 Continued

Kim SY 2014	$PM_{2.5}$ exposure and chemical composition of $PM_{2.5}$ exposure	CAC 48.9% had CAC >0, and their mean CAC score are 61.9 (SD=10.1); Two scans were obtained for each ID; the mean Agaston score of the 2 scans were used	Relative risk	NS	Age, gender, race/ethnicity; Framingham Risk Score (total cholesterol, HDL cholesterol, smoking status, hypertension, lipid-lowering medication); extended set of variables (education, income, waist circumference, body surface area, BMI, BMI2, diabetes, LDL, triglycerides); metropolitan area	Effect modification by variables not assessed
Dorans 2016	$PM_{2.5}$ exposure and residential proximity of major roads	CAC CAC was detectable in 47% of observations Detectable CAC progression thresholds are: CAC=0, threshold change in score of 3.4; 0<CAC≤100, change of 15.9; 100<CAC≤300, change of 46.7; 300<CAC≤1000, change of 73.7; CAC>1000, change of 325	ORs of: -Detectable CAC and average CAC -Detectable CAC progression and average annual change in CAC	NS	Primary model: age, age2, sex, BMI, smoking, education, median census-tract value of owner-occupied housing units (quartiles), cohort (offspring or third generation), date of scan and number of days between scan and examination at which individual-level covariates reported. Repeated measures analyses: scan (1st or 2nd round) ; for detectable CAC progression: age at MDCT1 (age, age2), covariates reported at Offspring Examination 7 or Generation 3 Examination 1, and time between MDCT scans; for annual change in CAC, age at 1st scan (age, age2) and time since first scan and included interaction terms with time since first scan: age at first scan (age, age2), sex and cohort	No effect modification by age, sex, cohort, 10-year risk of atherosclerotic CVD, or smoking status was found

Table 3.2 Continued

Kaufman n 2016	$PM_{2.5}$ exposure	CAC Two chest CT per participant, calculated mean Agatston score; CAC increased average by 24 Agatston units per year (SD 58)	Agatston unit yearly change per IQR (5μg/m^3) increase	4.1 (95% CI: 1.4 to 6.8)	baseline age, sex, ethnicity, site, CT scanner type, BMI, physical activity, smoking and second-hand smoke exposure (both time-varying), employment outside the home, total cholesterol, HDL, triglycerides, statin use (time-varying), neighborhood socioeconomic index, level of education, income Additional models: blood pressure and diabetes.	Effect modification by several factors was weak and inconsistent between $PM_{2.5}$ and NOX, another exposure this study evaluated Suggested a greater impact to elderly (65-74 years), women, non-obese (BMI ≤ 30), hypertensive individuals
Wang 2019	$PM_{2.5}$ exposure	CAC Summed total CAC score Mean CAC was 91.4 (322.2) Agatston units	% increase per IQR increase (30 μg/m^3)	27.2 (95% CI: 10.8 to 46.1)	Age, sex, BMI, smoking (status, duration, and intensity), alcohol consumption, education, and physical activity; area-level variables: urbanization (2500 population per 1 × 1-km^2 grid), study region (ie, north, southeast, and southwest), Beijing residence (yes or no), and categories of residence distances to Fuwai Hospital	Potential effect modification was evaluated for specific participant characteristics (age and sex), disease risk factors (BMI, smoking, diabetes, and statin use), and geography (region and urbanization) Association of CAC with $PM_{2.5}$ was greater among male, diabetes, and elderly (>= 60 years) participants In restricted analyses, postmenopausal **women** (N= 1732 out of 3790, 45.7%), had an even stronger association between $PM_{2.5}$ and CAC ($PM_{2.5}$ per 10 μg/m^3: 34.5%; 95%CI, 5.8% to 70.9%)
	$PM_{2.5}$ exposure	CAC>0 -> detectable CAC	OR of detectable CAC per 1 unit increase in $PM_{2.5}$	1.28 (95% CI: 1.13 to 1.45)		

Table 3.2 Continued

$PM_{2.5}$ exposure	CAC>400-> severe CAC	OR of severe CAC	1.59 (95% CI: 1.20 to 2.21)

$PM_{2.5}$, particulate matter ≤2.5 μm in diameter
OR, odds ratio; RR, relative risk; IQR, interquartile range;

Table 3.3 Effect modification for the association between $PM_{2.5}$ exposure and the development of CIMT

Author, year	Study Design	Study population	Pop size #	Exposure	Outcome	Evaluated effect modification
Kunzli, 2005	Cross-sectional	Two clinical trials' participants from Los Angeles, CA, USA, 1998-2003	798	$PM_{2.5}$	CIMT	For CIMT, association between $PM_{2.5}$ and CIMT was **stronger among older (≥60 years), women,** person using lipid-lowering therapy at baseline, and never smokers. Strongest association was in **women ≥60** years (N=186 out of 355 women) (15.7%, 95% CI: 5.7-26.6%)
Diez Roux, 2008	Cross-sectional	USA, 2000-2002 MESA	4912	$PM_{2.5}$	CIMT	The following variables were explored for effect modification: age, sex, lipid levels, site, education, race/ethnicity, diabetes, BMI, smoking. **No** effect modification by age or sex was found
Kunzli, 2010	Cohort, Longitudinal analyses	Five clinical trials' participants from Los Angeles, CA, USA, 1998-2003	1483	$PM_{2.5}$	CIMT progression	Associations between $PM_{2.5}$ and CIMT progression were **not different** by sex, lipid-lowering treatment, and ethnicity.
Bauer, 2010	Cross-sectional	Germany, 2000-2003 HNRS	3380	$PM_{2.5}$	CIMT	Effect modification was investigated by including interaction terms, and no effect modification by sex was found. Younger and obese participants, those without diabetes mellitus, statin users, and participants with any full-time employment and residents of Bochum and Essen, Germany show slightly stronger associations, but all 95% CIs were not statistically significant.

Table 3.3 Continued

Adar, 2013	Cohort, Longitudinal and cross-sectional analyses	USA, 2000-2002 MESA	N=6256 for baseline; N=5660 (83%) had 2 ultrasound exams, 2000-2006; N=5362 had no missing data	$PM_{2.5}$	CIMT risk and progression	Effect modification was examined by age, gender, race, education, obesity, diabetes, hypertension, statin therapy, and baseline CIMT Association between $PM_{2.5}$ and CIMT was strong among **women,** diabetics, hypertensive, and residents of St Paul.

CIMT: carotid intima-media thickness test

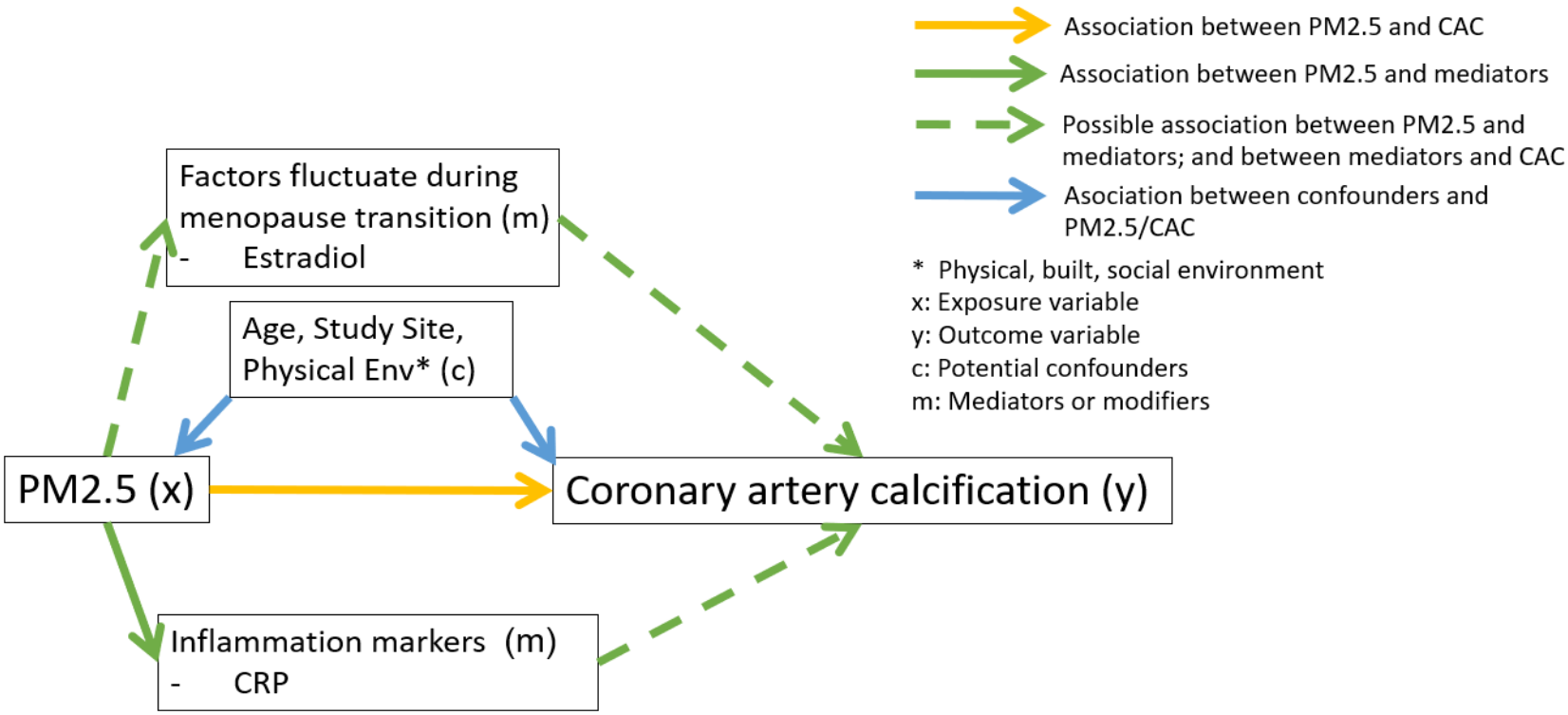

Figure 3.1 Conceptual model for assessing the association between $PM_{2.5}$ and CAC in women transitioning through menopause

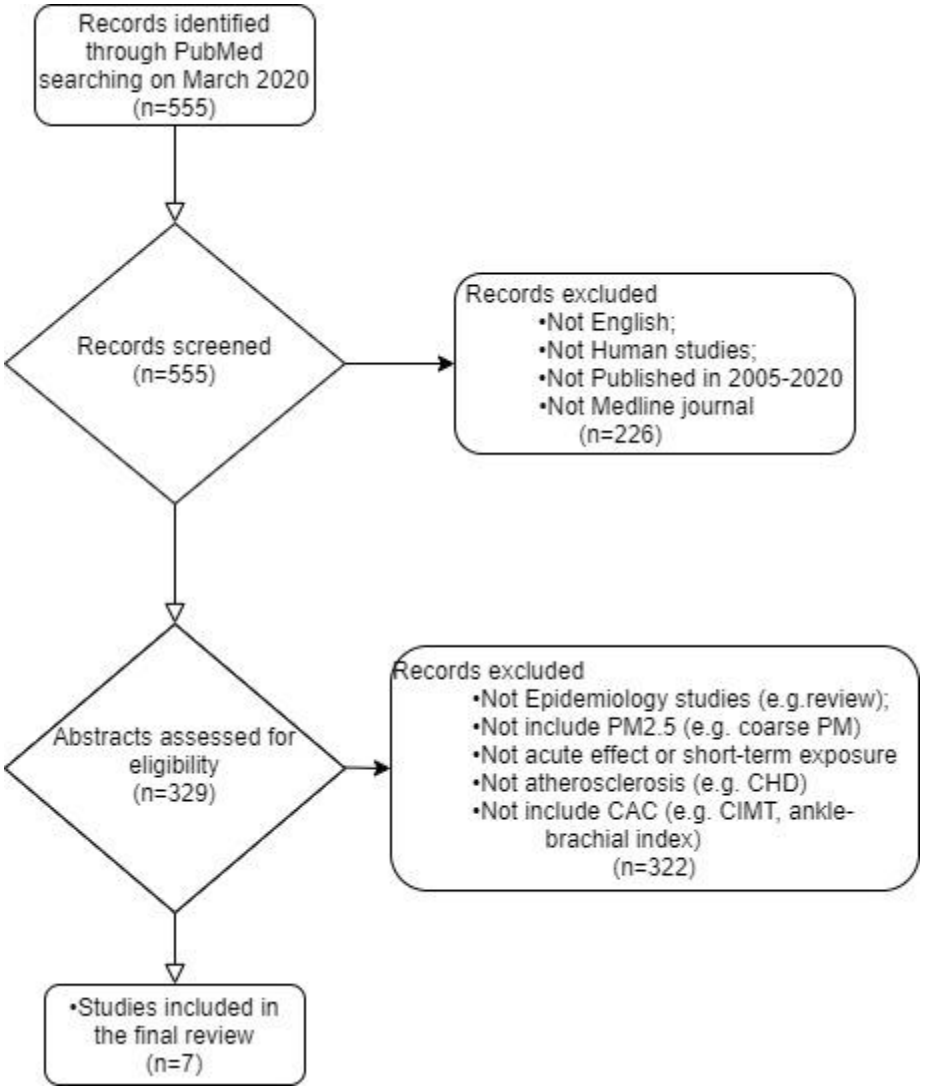

Figure 3.2 Inclusion and exclusion criteria for literature review on previous studies evaluated association between $PM_{2.5}$ and CAC

4.0 Is There an Independent Effect of Particulate Matter ($PM_{2.5}$) Air Pollution and the Development of Coronary Artery Calcification in Middle-Aged Women with Polycystic Ovary Syndrome?

4.1 Abstract

Objective

To assess the association of $PM_{2.5}$ ambient air pollution (AP) and coronary artery calcification (CAC) in middle-aged women with and without polycystic ovary syndrome (PCOS).

Background

The relationship of long-term AP and SCA (subclinical atherosclerosis) has been studied in several cross-sectional and prospective investigations. The association, however, has yet to be studied among middle-aged women with PCOS. PCOS, a heterogeneous disorder with developmental origins and which affects approximately 10% of women of reproductive age is characterized by chronic anovulation, hyperinsulinemia, hyperandrogenemia and increases in cardiovascular risk factors. We hypothesize that women with PCOS may be more susceptible to the effects of AP on the risk of SCA.

Methods

A case-control design was originally adopted to study the relationship of SCA and PCOS. We employed the same design to determine if there is an independent effect of long-term AP exposure on the prevalence of SCA in women with PCOS compared to its control group. Long-term exposure was defined as the annual average of $PM_{2.5}$ for the year of the baseline visit based on residential history. US EPA hierarchical Bayesian modeled air pollution levels for 2001-2003

were assigned to the 136 cases with PCOS and 165 population-based controls. These women had originally been enrolled in phase III of the Cardiovascular Health and Risk Measure (CHARM) study in Pittsburgh, PA. The outcome was CAC Agatston score, as measured by electron beam computerized tomography (EBCT). Logistic regression models were used to estimate the association between $PM_{2.5}$ and CAC (categorized into: CAC<10 vs. CAC≥10). Relationships between $PM_{2.5}$ and CAC were studied for all women, as well as among women with PCOS and their control groups, separately. Effect modification of PCOS and $PM_{2.5}$ was added into the final model to explore its role to the association.

Results

Overall, the participants had mean age of 47.9±5.7 years; and the annual mean $PM_{2.5}$ levels was higher in women with CAC≥10 compared to <10 [17±1.2 vs. 16.2±1.5 $\mu g/m^3$, p=0.008]. Women with PCOS had a comparable annual mean $PM_{2.5}$ level to that in women without PCOS [16.4±1.4 $\mu g/m^3$ vs. 16.7±1.2 $\mu g/m^3$]. In adjusted analysis for age, BMI and PCOS, using combined data in both cases and controls, long-term $PM_{2.5}$ exposure was not significantly associated with CAC measures, but the association were significantly modified by PCOS status (p for interaction =0.02). When PCOS cases and controls were considered in separate models, there was a significant relationship between long-term $PM_{2.5}$ exposure and CAC among PCOS cases (N=136) in adjusted model (OR=1.44; 95% CI: 1.02, 2.04). There was no relationship noted between $PM_{2.5}$ exposure and CAC among women without PCOS.

Conclusions

$PM_{2.5}$ exposure was associated with CAC risk in middle-aged women with PCOS, but not in the women without PCOS. There was a non-significant effect of $PM_{2.5}$ among women without

PCOS. These findings suggest PCOS as a potential modifier of how AP might be related to CAC risk. Further studies in larger groups of women with PCOS is needed to confirm the current finding.

Key Words: $PM_{2.5}$, air pollution, subclinical atherosclerosis, coronary artery calcification, polycystic ovary syndrome, middle-aged women

4.2 Introduction

Fine particulate matter <2.5 mm ($PM_{2.5}$) air pollution is the most important environmental risk factor that contributing to cardiovascular mortality and disability worldwide (1). Looking specifically at cardiovascular disease (CVD), air pollution was responsible for 19% of all cardiovascular deaths globally in 2016 (190). Women as compared to men are at increased risk of elevated levels of subclinical coronary atherosclerosis (SCA) and CVD (17–19). The goal of this work is to assess the theory that the effects of $PM_{2.5}$ on atherosclerosis are more apparent and/or stronger in middle-aged women. As an important subpopulation, middle-aged women with polycystic ovarian syndrome (PCOS) have greater underlying risk of CVD; and hence may demonstrate even stronger associations of $PM_{2.5}$ effects on atherosclerosis.

PCOS is a prevalent condition affecting 10% of reproductive aged women (191). Recent investigations have considered this group as a particularly vulnerable population to the adverse effects of air pollution and further explored the effect of $PM_{2.5}$ on female morbidity and mortality among women with PCOS (155,156), which could be the interplay effect of genetic and environmental factors. One of these investigations is an observational study conducted in Taiwan, China to assess the association between $PM_{2.5}$ exposure and PCOS (Total number of PCOS=2072, after 12 years of follow-up). The study reported that $PM_{2.5}$ exposure is associated with an increased

risk of PCOS (increased by 3.56, 95% CI: 3.05-4.15) (156). The data however, was applying residential history from the present to the risk of developing disease. PCOS however, has onset at puberty or earlier making the conclusion fallacious based on where they were living in adulthood or older age. There is no strong evidence except for one study that did indicate that air pollution could exacerbate symptoms related to menstrual irregularity, and may affect infertility (155). Our paper proposed that PCOS women could be more susceptible to air pollution effects to SCA was based on the consideration of long-term $PM_{2.5}$ exposure as a potential endocrine disrupting chemical (EDC) that may affect PCOS. A study has suggested that prolonged exposure to EDCs through early life could potentially result in hormonal destabilization and may further lead to metabolic alterations that can exacerbate the PCOS and contribute to consequences such as CVD (192). The evidence from this study indicates that PCOS might potentially be early sequelae of long-term air pollution exposure, though future experimental investigations are needed to explore this. Additionally, studies have shown that many air pollutants (e.g. $PM_{2.5}$) could potentially interfere in the functioning of the endocrine system and may play a role as EDCs (193,194), which constructs a foundation for our posed hypothesis.

Women with PCOS are an unique population due to their significant metabolic derangements, including type 2 diabetes, insulin sensitivity and hyperandrogenemia (195). Compared with women without PCOS, women with PCOS have an increased adverse cardiovascular risk profile, including higher levels of low-density lipoprotein cholesterol (LDL-C) and triglycerides, increased abdominal adiposity, and higher systolic blood pressure (122,123,131,135). These characteristics have been associated with greater coronary artery calcification (CAC) risk (41,196). The biologic mechanisms linking these abnormalities to $PM_{2.5}$ toxicity are still a subject of research. One potential pathophysiological pathway is that inhaled

$PM_{2.5}$ might directly trigger arrhythmia, blood coagulation or vascular dysfunction, and the other potential way is that $PM_{2.5}$ could stimulate inflammation or mediate systemic and possible blood pressure increase, which could further influence the development of atherosclerosis (96–98,100). Furthermore, it has been demonstrated in animal studies that long-term exposure to ambient $PM_{2.5}$ leads to both endothelial dysfunction and accelerates the progression of atherosclerosis, especially among mice deficient in apolipoprotein E or low density lipoprotein receptor, which develop advanced atherosclerotic lesions (99). Those indicate that air pollution may accelerate the development of coronary atherosclerosis and progression of atherosclerosis over time, through one or more of those above-mentioned pathways.

Whether the association between $PM_{2.5}$ exposure and CAC differ among middle-aged women with or without PCOS is an open question. PCOS as a diagnosed condition was well measured in a case control study conducted between 1994-2006 that serves as the basis for the current analysis. The CHARM study conducted by Talbott et al (Cardiovascular Health and Risk Measurement Study (NIH RO1440660) originally recruited participants in 1994-1997 in the greater Pittsburgh area (197). Pittsburgh is a U.S. city consistently ranked in the top 10 for air pollution and remains among the worst in the country (198). The results of the previous CHARM investigation showed that lifelong exposure to an adverse cardiovascular risk profile (e.g. insulin sensitivity, abnormal glucose, low HDL) in women with PCOS leads to premature atherosclerosis (130). In addition, another study using data from CHARM reported that women with PCOS have greater amounts of CAC and lower vasodilation (a measure of endothelial function) than in women without PCOS (138), consistent with another finding from a study by Talbott et al., 2008 (73). Therefore, our research question was: do women with PCOS have a significant association of

$PM_{2.5}$ exposure with coronary artery calcification independent of other risk factors compared to a control group of non-PCOS women?

Our main objective was to evaluate the association of $PM_{2.5}$ exposure with CAC risk in middle-aged women with and without PCOS using the CHARM III study data, 2001-2003. The participants of CHARM III study had data on atherosclerosis measured by CAC Agatston score, PCOS status, cardiovascular risk profiles and $PM_{2.5}$ exposure. We hypothesize that women with higher levels of $PM_{2.5}$ exposure is associated with an increased risk of CAC independent of other cardiovascular risk factors during their midlife. Moreover, the effect of $PM_{2.5}$ on CAC is stronger among middle-aged women with PCOS compared to those without.

4.3 Methods

Study Participants

A series of investigations began in 1994 that studied cardiovascular risk factors in women with PCOS. The first study was conducted in the Pittsburgh area, and included 244 women with PCOS aged 19-55, and 244 age and race matched controls (197). Control subjects were selected in the parent study using a voter registration tapes from the greater Pittsburgh area and Cole's Cross Reference Directory of Households (Cole Publications, Lincoln, N3). This study determined that women with PCOS had significant increase in cardiovascular risk factors after adjusting for age and BMI than controls (197). The two studies that followed the original investigation were conducted in 1997-1999 and in 2001-2006, with a focus on subclinical atherosclerosis. The first one focused on carotid intima-media thickness (IMT) and the second on CAC and included an older aged subset of these women who were 35 years of age and older plus an additional group of

minority women in an attempt to increase the racial heterogeneity of the study population (130,138). As those two later investigations focused on subclinical atherosclerosis measures, the age was set at 35 that reduced the number of subjects available for follow up and resulted in breaking the original match (130,138). A minority supplement also added additional PCOS cases and controls. Details on the identification and recruitment of the original Pittsburgh PCOS cohort have been reported elsewhere (73,197). This parent study obtained detailed information on cardiovascular risk factors in the group with largest number of PCOS cases by that time (73).

For the current analyses, we restricted our study sample to cases and controls who participated in the phase III follow-up study of CHARM, CHARMIII. The analytical sample size included 301 women (35+ years of age) who had valid CAC and $PM_{2.5}$ exposure data available (73,138), including 136 PCOS cases and 165 controls. All the measures were taken at the time of the baseline CHARMIII visit that occurred between 2001 and 2003. The Institutional Review Board of University of Pittsburgh approved this study (IRB number: 0401091). Written informed consents were obtained for all the participants. The detailed data reduction figure was presented in the Figure 4.1. Compared with those included women, the excluded women were younger, and with higher BMI. Among those included women, there were 136 PCOS cases and 165 controls, included in our analyses.

Exposure to $PM_{2.5}$ assessment

The air pollution data were obtained from the U.S. Environmental Protection Agency (EPA) database. The predictions of ambient $PM_{2.5}$ were obtained using a downscaling modeling approach (19).This downscaling model used a Bayesian space-time modeling to combine air monitoring data and gridded numerical output from the Community Multi-Scale Air Quality Model (http://epa.gov/asmdnerl/CMAQ) to produce daily prediction (19,199). Further modeling

details and results from this model can be found at the U.S. EPA website (19). Daily predictions of $PM_{2.5}$ from January 1, 2001 to December 31, 2003 at the geographic centroid of each 2000 US Census ZCTA was obtained using the above-mentioned modelled data. These daily values were used to estimate an annual average value of $PM_{2.5}$. Exposure to $PM_{2.5}$ annual average estimated values were linked to each participant's residence address of ZIP code centroid at time of clinic visit. The annual exposure of $PM_{2.5}$ were defined as exposed at the CHARMIII CAC scan visit date. These data have been used and validated in several studies, including the investigation of acute myocardial infarction and hospitalizations by Talbott et al. (19).

Coronary artery calcium assessment

CAC was assessed in 2001-2003 at the baseline visit at the University of Pittsburgh Medical Center (UPMC) Preventive Heart Care Center using the Imatron C-150 Ultrafast CT scanner (Imatron, South San Francisco, CA, USA) (73). The CT scans were conducted at the Heart Institute by Dan. Edmundowicz, MD. Detailed data collection and methodologies of CAC measurements have been published previously (73,138). CAC scores were generated using a Base Value Region of Interest (BVROI) computer program (AcuImage), which extracts all pixels above 130 Hounds field units within an operator-defined region of interest in each 3mm thick image of the coronary arteries. All pixels greater than 130 Hounds field units and larger than one mm^2 within the coronary arteries were considered calcium. A CAC score was then calculated for each region of interest by multiplying the area of all significant pixels by a grade number (1,2,3 or 4) indicative of the peak CT number (Hounds field units) (Agatston scoring method) (63). The individual region-of-interest scores were then summed for a total CAC Agatston score (63). In order to minimize radiation exposure to the study participants, only one scan was performed per visit. We defined the participants with valid CAC scores for inclusion criteria as those with: 1) age of 35-64

years old; 2) BMI of 18.5-49.9 km/m^2; 3) race of Caucasian or African American only; and 4) out insulin dependent diabetes mellitus (IDDM) or type-1 diabetes. We categorized CAC into: 1) CAC<10 Agatston units; and 2) CAC≥10 Agatston units (70). These cutoffs of presence of CAC have been widely used in the previous study (70), as the low values (<10) of CAC are not reliably quantitated and women at low or moderate risk of CVD could have CAC scores fall in this range.

PCOS status of participants

A clinical diagnosis of PCOS was established at baseline of the parent study if there was 1) a history of chronic anovulation in association with either 2) evidence of clinical and/or biochemical hyperandrogenism (hirsutism or total testosterone level greater than 2.0 nmol/liter, respectively) or 3) a luteinizing hormone (LH) and follicle-stimulating hormone (FSH) ratio greater than 2.0 (130).

Study variables

Risk factors' measures and demographic characteristics data of those women are collected, including information on anthropometrics, blood pressure, lipid levels, hormone levels, and fasting glucose and insulin levels.

Natural menopausal status was defined as not having a period for 12 months or more when there was no medical cause other than menopause with or without hormone replacement therapy (73). Those who reported a hysterectomy with bilateral salpingo-oophorectomy (BSO) were classified as surgical menopausal (73). In addition, women who reported removal of the uterus with one or two ovaries intact were classified as surgical if they reported a history of physician prescribed hormone replacement therapy or had a biochemical profile of the reproductive hormones indicative of menopause (73). This consisted of a FSH level greater than 25 mIU/ml, and a progesterone level below 3 ng/ml (73). This model was adapted from the algorithm applied

in the WISE study (Women's Ischemia Syndrome Evaluation) (200). Individuals who did not meet these criteria were considered as in premenopausal status. Due to the small sample size, we grouped surgical menopausal and women with drug therapy (to damage the ovaries and cause menopause) together into a category and coded it into missing, and this is because our main interest is to compare the natural menopausal to premenopausal women.

Detailed demographic and disease characteristics' data collection and laboratory methodologies have been published previously (73,197). In brief, height was measured to the nearest half inch on a wall mounted Harpenden stadiometer; weight was measured to the nearest half pound; and BMI (kg/m^2) was calculated using those measured height and weight. Waist and hip circumferences (in cm) were measured in duplicate with an inelastic tape at the level of the umbilicus and greater trochanter, respectively; the average value was recorded for calculating the waist: hip ratio. Blood pressure was assessed in duplicate after a 30-minute caffeine restriction and 5-minute rest using a random-zero sphygmomanometer. A questionnaire was administered that included the evaluation of medical (e.g. hypertension, type 2 diabetes), menstrual and reproductive histories (e.g. HRT use, menopause status), current medication use (e.g. insulin taking, hypertension medication taking), lifestyle factors (e.g. current smoking, occasion to drink), and family history of PCOS (e.g. familial PCOS). In addition, self-reported race, age, education, and residential address with zip codes were collected at baseline screening, 2001-2003. (130)

A 12-hour fasting blood sample was obtained and serum concentrations of total cholesterol, HDL2 (not HDL-C), LDL-C, and triglycerides were measured in the Heinz Lipid Laboratory at the University of Pittsburgh (73). Total cholesterol was determined by the enzymatic method of Allain et al (1974) (201). HDLT was determined by the method above after selective precipitation by heparin/manganese and the removal by centrifugation of VLDL and LDLT (73). LDLc was

calculated using the Friedewald formula (202). Triglycerides were determined using the enzymatic procedure of Bucolo and David (1973) (203). Plasma glucose was analyzed by using enzymatic assay (Yellow Springs Glucose Analyzer, Yellow Springs Instruments) and plasma insulin by radioimmunoassay. History of hypertension was defined as either were taking hypertension medication, or had systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg. Type 2 diabetes history was defined as either a doctor diagnosis of Type 2 diabetes or a fasting blood glucose of >125 mg/dl. The detailed measurement methods of each of the above covariates were described previously (73,138). All of the covariates were obtained at the same time that the EBCT was conducted during this 2001-2003 clinical assessment.

Statistical analyses

The distribution of CAC was markedly skewed and could not be normalized using traditional mathematical transformations. Hence, the CAC was categorized into two categories: 1) CAC<10; and 2) CAC≥10 (70), and logistic regression modelling was used to test the association between $PM_{2.5}$ and CAC among all women, and among women with PCOS and without PCOS. Bivariate analyses were conducted using T tests or Wilcoxon-Mann-Whitney tests for all continuous variables depending on whether those variables are normally distributed or not, by PCOS status and by categorized CAC. As the CHARMIII is a PCOS case-control study, we present the descriptive statistics at baseline (2001-2003) by PCOS status first, and then stratify by categorized CAC for both women with and without PCOS groups. Additionally, Chi-square tests or Fisher's exact tests were performed for all categorical variables, by PCOS status and by categorized CAC. Non-normally distributed continuous data were logarithmically transformed before performing statistical modelling (e.g. triglyceride levels).

Univariate regressions of CAC with specific cardiovascular risk factors were conducted to identify the independent baseline cardiovascular risk factors that predicted CAC as the dependent variable. In the multivariable logistic regression models, the effect of $PM_{2.5}$ on CAC independent of age, PCOS, and those cardiovascular risk factors found to be significant in the univariate regression analyses or were potential confounders was assessed. More specifically, age, PCOS, and BMI were a priori selected covariates and were forced into the base model regardless of statistical significance. All other variables that were found to be significantly associated with cardiovascular risks and independent variables in the univariate analyses were considered as potential covariates to be adjusted for in the adjusted models. Cardiovascular risk factors explored in these analyses included BMI, waist-to-hip ratio, systolic and diastolic blood pressure, LDLc, HDL2, triglycerides, fasting insulin, smoking status, drinking status, and hormone use. We then added the selected covariates as above described into the models. However, due to the small sample size, we added one covariate into the model each time, along with PCOS, age and BMI (the priori-selected covariates) to avoid overfitting issue. Those cardiovascular risk factors would be confounders of the potential mediator (PCOS) to outcome (CAC), but would not be confounders of the exposure ($PM_{2.5}$) to outcome (CAC) relationship. Interactions of $PM_{2.5}$ and PCOS status were also created and were fit into models to assess whether PCOS is effect modifier. Furthermore, subgroups analyses by PCOS status were conducted to assess the association between $PM_{2.5}$ and CAC among women with and without PCOS.

All statistical analyses were performed using SAS 9.4 (Cary, North Carolina) or Stata 16 (StataCorp, College Station, TX). Two-sided p-values ≤ 0.05 were considered statistically significant.

4.4 Results

Descriptive results of sociodemographic and cardiovascular risk factors in PCOS cases and controls are presented on 301 women by PCOS status (PCOS N=136; and Non-PCOS N=165) who met our previously stated criteria in Table 4.1. Participant characteristics, including smoking status, hormone use, anthropometric measures, blood pressure, lipids, and insulin, at the time of baseline EBCT (2001–2003) are presented in Table 4.1 for 136 PCOS cases and 165 controls. The $PM_{2.5}$ exposure annual mean levels were similar between women with PCOS and without (p=0.09). PCOS women had more CAC presence (CAC≥10) compared with controls (33.8% vs. 13.9%, p<0.0001). The median CAC score was 3.09 for women with PCOS (Q1-Q3: 0-16.0), compared to zero for women without PCOS (Q1-Q3: 0-3.4) (p<0.0001). Additionally, more controls than women with PCOS were either surgical or natural postmenopausal. PCOS women had higher mean BMI compared to control women (31.7 vs. 28.3 kg/m^2) and p<0.0001. The triglyceride levels and insulin levels were higher among women with PCOS, compared with the controls (p=0.029 and <0.0001, respectively). (Table 4.1).

With recognizing that there are clear differences in cardiovascular risk factors in women with and without PCOS, we also presented the demographic and clinical characteristics of these two groups by CAC categories. We dichotomized CAC categories at the baseline visit: CAC< 10 vs. ≥10. PCOS cases and controls' characteristics by presence of CAC (<10 vs. ≥10) are presented in Table 4.2. The detailed descriptions of these variables are included in the Supplement material.

Univariate logistic regression of CAC with selected demographic characteristics and cardiovascular risk factors are shown in Table 4.4. Neither race nor education were significantly associated with CAC presence. Cardiovascular risk factors which were related on a univariate basis to greater CAC included: PCOS status, BMI, DBP, SBP and HDL_2, as well as triglycerides,

glucose, and insulin levels (Table 4.4). $PM_{2.5}$ upon univariate analysis for the total case control group was not significant.

Table 4.5 presents the association between $PM_{2.5}$ (1 $\mu g/m^3$) exposure and CAC presence, by the selected cardiovascular risk factors for the case control population of 301 women. For each of the models, the individual covariates entered in a stepwise fashion. $PM_{2.5}$ was elevated but did not research significance (confidence limits reaching 1.00 in the lower most boundary). The multivariable logistic regression models were carried out to assess the independent effect of $PM_{2.5}$ on CAC, adjusted for confounders of age, PCOS, and BMI, and the effect size of $PM_{2.5}$ was borderline positive associated with CAC, though it was not statistically significant (OR=1.17; 95% CI: 0.91-1.50). All the other results are presented in Table 4.5. Subsequent addition of adjusting for the other cardiovascular risk factors to assess each of their effect on the $PM_{2.5}$ and CAC association in separate models did not alter the $PM_{2.5}$-CAC association.

In the subgroup analyses, we further evaluated the association between $PM_{2.5}$ levels and CAC risk among women with PCOS and among women without PCOS (Table 4.6 and 4.7). Among women with PCOS, there was an obvious positive association between $PM_{2.5}$ and CAC (CAC<10 vs. CAC≥10 (OR=1.44; 95% CI: 1.08, 1.92), adjusted for age (Table 4.6). When we further adjusted for other cardiovascular risk factors (e.g. LDL, BMI), the association remained the same and still significant (Table 4.6). Among women without PCOS, there was no statistically significant association between $PM_{2.5}$ and CAC in any of the tested models with or without adjusting for confounders (Table 4.7). Further analyses adjusting for other cardiovascular risk factors change neither the effect size of the association nor the significance. However, BMI remained a significant predictor of CAC among all those tested variables (Table 4.7).

Furthermore, an interaction term of $PM_{2.5}$ and PCOS was also entered in the model and indicated that PCOS is an effect modifier (p for interaction in the adjusted model=0.02) (Table 4.8). To explore the possible reason for the negative coefficient of the interaction term (Table 4.8), we generated scatterplots for $PM_{2.5}$ versus continuous CAC score for PCOS cases and controls separately (Appendix A). We plotted continuous CAC as it is hard to discern patterns using dichotomous CAC. The CAC outliers in the cases and controls were removed prior to the scatterplots. The figures show that the CAC score is positively related to $PM_{2.5}$ among women with PCOS group and the controls appear to be in the negative direction, explaining the negative beta coefficient in the interaction results. $PM_{2.5}$ and CAC score appear to have a flat trend for women without PCOS group which reveals a non-significant negative coefficient as compared to the women with PCOS group which reveals a positive coefficient.

4.5 Discussion

Among women with PCOS, there was a significant association bewteen long-term $PM_{2.5}$ and an increased risk in CAC presence. Such an association was not detected among women without PCOS. With both PCOS cases and controls in the model, the odds ratios of CAC affected by $PM_{2.5}$ exposure although increased for each of the tested models, they did not reach statistically significance. Our study found that PCOS is an effect modifier of this association in this cohort of middle-aged women.

Our findings of no association of CAC measures with long-term $PM_{2.5}$ among the middle-aged women overall are in agreement with most of the prior studies (8,9,11–13). Among the observational studies which assessed the association between ambient air pollution ($PM_{2.5}$) and

CAC, the mean level of $PM_{2.5}$ exposure among the U.S. MESA (Multi-Ethnic Study of Atherosclerosis cohort), the German Heinz Nixdorf Recall Study (HRNS), and the Framingham Offspring cohort studies ranged from 13.7 to 26.7 $\mu g/m^3$ (8–13). $PM_{2.5}$ exposures of our study (mean=16.6, SD=1.3 $\mu g/m^3$) are also in the same range at lower end as the previous studies, making our studies using different population comparable for assessing the association. No association between $PM_{2.5}$ exposure and CAC were reported by most of those studies, including the German cross-sectional study of the HRNS, three cross-sectional studies of the MESA, and a longitudinal study using the Framingham Offspring cohort (8–14). Our results add to the body of this literature with the ability to consider the potential effect of $PM_{2.5}$ on SCA, especially in middle-aged women with one group as a control and the other women who are more metabolically challenged.

We demonstrated that there is a significant association between $PM_{2.5}$ exposure and CAC in middle-aged women with PCOS, but no such an association detected among women without PCOS. To the best of our knowledge, two studies, the U.S. MESA by Kauffman et al., 2016 and the Chinese Coronary Atherosclerosis Disease Early Identification and Risk Stratification by Noninvasive Imaging (CREATION) study by Wang et al., 2019, reported a positive association between $PM_{2.5}$ exposure and CAC among general population and among postmenopausal women (10,14). The average $PM_{2.5}$ exposure was 70.1 $\mu g/m^3$ (SD=20.0) in the CREATION Chinese study, which is higher than the exposure of our study. As mentioned, the exposure levels of our study are similar to the U.S. MESA study. Our study and those above-mentioned two studies used a more reliable and valid modelling for estimating the air pollution levels (e.g. hierarchical Bayesian modeled $PM_{2.5}$ exposure) than the ordinary kriging or monitor data (31). For the future research evaluating the association in larger population to confirm our findings, using the more reliable

modelling estimated air pollution data other than monitor-based data will be critical to obtain the accurate measures.

In the current study, we found that there is a significant interaction between PCOS status and $PM_{2.5}$ exposure on the association of $PM_{2.5}$ and CAC presence. There were no any previous observational studies conducted to assess the association of $PM_{2.5}$ exposure and CAC among women with PCOS. Our finding indicates that women with PCOS might be impacted by long-term $PM_{2.5}$ exposure more to develop coronary atherosclerosis. This is what we have been hypothesized originally based on the previous findings that women with PCOS is associated with a higher risk of atherosclerosis and cardiovascular risk than women without. Previous studies concluded that women with PCOS may represent the largest subgroup of the female population at high risk for early sub-clinical atherosclerosis and subsequent clinical cardiovascular disease (137). A growing number of epidemiologic studies have reported that women with PCOS have substantially greater amounts of coronary calcification and CVD (73,130–132,134,138,139). Additionally, the major CVD risk factors appear to be more common among women with PCOS, including, obesity (increased central adiposity or BMI, or waist-to-hip ratio), insulin resistance (hyperinsulinemia), dyslipidemia (elevated LDL or triglycerides), and type 2 diabetes (131,132,134–136). A previous study also concluded that there was a significantly higher rate of detectable CAC among women with PCOS compared with controls (54% vs. 24%, $p< 0.05$) (73). Our findings on the positive association between $PM_{2.5}$ and CAC only among women with PCOS, but not among the controls are in line with those previous studies.

With all those findings reported by the previous studies mentioned above, it is clear that the PCOS women are at higher risk of SCA. However, the pathophysiology of PCOS and CVD that relevant to $PM_{2.5}$ toxicity is still unclear. There is limited research as to how PCOS might

affect the association between $PM_{2.5}$ and CAC. Studies have shown that there is an association established between PCOS and CHD, and PCOS has been studied as a risk factor of the increased risk of CVD (131,132,134). However, limited studies have shown that air pollutants (e.g. $PM_{2.5}$) might potentially interfere in the functioning of the endocrine system and may play a role as EDCs (193,194). Additionally, a study indicates that long-term exposure to $PM_{2.5}$ as a potential EDC from early life might be related to destabilization of hormonal homeostasis and may further lead to metabolic alterations that can exacerbate the PCOS and further contribute to consequences such as CVD (192). Even though the pathophysiology underlying what might be going on with PCOS and $PM_{2.5}$ is not still clear, those above-mentioned evidences serve the bases for our study to consider as potential mechanism linking air pollution and cardiovascular conditions among women with PCOS. Future studies are needed to explore whether $PM_{2.5}$ plays a role as EDCs to the development of PCOS or CAC, and if this potential link of long-term $PM_{2.5}$ exposure and CAC through PCOS is plausible in an experimental setting. The timing of $PM_{2.5}$ exposure is crucial to pay attention to for studying SCA development among women with PCOS, especially in the early development periods, as exposure to EDCs that mimic endogenous hormones may contribute to PCOS development and adverse CVD health effects. Our findings, along with those above-described studies' results will serve as a base for future studies.

In a cross-sectional study by Christian et al. using a group of premenopausal women (aged 30-45 years), they concluded that women with PCOS have a greater prevalence and extent of coronary calcification than in obese or non-obese women with similar age women and obesity increases the risk of CAC in those premenopausal women with PCOS (140). Furthermore, studies have also reported that among women with PCOS, BMI is a significant predictor of the increased CAC risk (138,140,141). Those above-mentioned findings suggested that the increased risk in

CAC among women with PCOS could be due to those factors/profiles that were different in women with PCOS compared with those without PCOS, including: elevated LDL-C, obesity, and hyperinsulinemia (138,140,141). Those findings served as a base for our additionally tested intermediaries when assessing the association between air pollution and CAC among women with PCOS. However, after we additional adjusted for each of those intermediaries (e.g. LDL, triglycerides, BMI, glucose), the effect size and significance of the $PM_{2.5}$ and CAC association remained the same, indicating that those factors do not add additional effect on the association in this cohort. Additionally, we added each of those cardiovascular risk factor variables in the model, one at a time and did not see any overall difference. We do need to be caution with this modelling approach that further adjusted for potential mediators, as it is not the optimal way for exploring the mediation analyses as doing so may block the pathway. In addition, this may bias results in terms of estimating a causal effect of $PM_{2.5}$ on CAC. However, both of our unadjusted model and adjusted models resulted in the similar effect sizes and remained the same significance for the tested associations.

Previous work has confirmd an increased risk in subclinical atherosclerosis (SCA) in women with PCOS compared to women without PCOS (138), but no one to date has considered the possible added effect of air pollution exposure to the development of SCA in this group of high risk women, like what we did. A study pointed out that when PCOS women reach the menopause, the second metabolic "insult" may be associated with a greater cumulative risk to an increased CVD and atherosclerosis (73). As there is little information available on their (women with PCOS) physiological changes during midlife, which may make the association between $PM_{2.5}$ exposure and atherosclerosis different in those women with PCOS than in women without, as what our study reported. Our study with the unique populations, women with PCOS and without PCOS, provided

a natural experiment for evaluation of the effects of hormonal abnormalities and other physiological changes and $PM_{2.5}$ on cardiovascular risk. This was why we conducted this study and compared this association among middle-aged women with and without PCOS, to understand the potential impact of PCOS on the association between $PM_{2.5}$ and CAC among midlife women. To conclude, we found that $PM_{2.5}$ is associated with an increased risk of CAC among women with PCOS but not among the controls.

To the best of our knowledge, there is no study investigated the association between $PM_{2.5}$ and CAC among women with PCOS. One observational study conducted to assess the association between $PM_{2.5}$ exposure and PCOS (Total cohort N=91,803; and No. of PCOS=2072 after 12 years of follow-up) (156). Although this study did not look at the CAC as outcome as it was not their study of interest. Their study provided evidence to suggest that the association might be more pronounced among women with PCOS. Even though they found positive association between $PM_{2.5}$ and PCOS, as mentioned earlier the rational of their tested association was questionable. Because the PCOS develops at puberty and is mostly inherited (genetic disorder) or effected by gene-environment interaction so that hypothesizing the PCOS might be caused by $PM_{2.5}$ as this study did is not valid. We, therefore, need to interpret their finding with cautions. Furthermore, a systematic review evaluated the effect of $PM_{2.5}$ to some symptoms that related to PCOS. They reported that there is a potential relationship between air pollution and infertility (PCOS is one of the most common cause of infertility), but no association detected between $PM_{2.5}$ exposure and endometriosis (it shares similarity with PCOS, e.g. both can result in irregular bleeding) (155). This is accordance with what we found that women with PCOS, who most likely had infertility and menstrual irregularity during their early life, were affected by air pollution exposure to increase their risk of CAC. The importance of estimating the association between $PM_{2.5}$ exposure

and women's health among PCOS population has been addressed by the meta-analysis. Our findings filled in this research gap and established the association between $PM_{2.5}$ and CAC among this unique population of women with PCOS.

Our study is the first study that focused on air pollution and CAC among middle-aged women with PCOS, which is a complex disorder of the endocrine system characterized by chronic anovulation, hyperandrogenism, and insulin resistance and is a common endocrine disorder of reproductive-aged women (122,123). Women with PCOS have metabolic abnormalities, including increased central adiposity, hyperinsulinemia, elevated LDL, triglycerides and glucose levels, low HDL levels, hypertension, and ultimately, adult onset Type II diabetes (131,135,136), making them at high risk for atherosclerosis. Hence, the additional exposure to $PM_{2.5}$ may make those women vulnerable during their midlife than general populations for atherosclerosis. This could explain our detected interactions between $PM_{2.5}$ and PCOS; and the positive association between $PM_{2.5}$ and CAC among only women with PCOS during their midlife. Although the statistical analyses of our interaction test supported that $PM_{2.5}$ and PCOS had multiplicative interactions on the association between $PM_{2.5}$ and CAC, we need to be aware that using statistical interaction to draw conclusions about biological interaction may not be appropriate. Our study is in observational design, therefore, conclusion on mechanisms of the biological pathway of the interaction effect on the association could not be drawn.

The study has several limitations. Because the study design and analyses are observational, there is potential for residual confounding. However, we have adjusted for several potential confounders during our modelling building process, and the effect sizes were similar and not statistically significant overall. In addition, we do not have detailed long-term follow-up information on participants' residential history. Because atherosclerosis is a chronic disease, and

takes time to progress, exposure over a long period might have a greater impact on the disease progress than the recent exposures. Furthermore, considering that our study sample size was relatively small (about 300 women), we need to interpret the findings with caution, especially for null findings. Our tests for interaction of $PM_{2.5}$ exposure and PCOS status on the association between $PM_{2.5}$ exposure and CAC, adjusted for confounders, found that PCOS is an effect modifier of this association in this cohort of middle-aged women. Replication of this work in other larger groups of women with PCOS would be needed to confirm our findings. Lastly, this study included women who are predominantly Caucasians; so those may not be generalizable to women of other ages or racial and ethnic groups.

There are several strengths of our study. This CHARMIII study population comprises one of the largest groups of women with PCOS. It is one of the few large-scale epidemiological case-control study (PCOS) in Pittsburgh, PA mostly, and has been followed for a decade to perform the CAC scans during their midlife for testing the association between PCOS and subclinical atherosclerosis (130). Since PCOS is most commonly diagnosed during women's reproductive lifespan (20-30 years old) when there is a wish to conceive, and their CVD normally manifests 30-40 years later. The role of PCOS on the association between $PM_{2.5}$ and CAC therefore has been difficult to study, because of the long latency period of CVD in women with PCOS, which are most likely determined by factors that arise in utero and in early life and play out over decades. Large long-term observational studies of well-characterized women with PCOS on this topic are rare, as it requires a long follow-up of women with PCOS from the premenopausal reproductive age until menopausal range (≥50 years) when there is greater cumulative risk to an increased CVD, as well as long-term chronic exposure of $PM_{2.5}$ to make impact on CAC. Our study has advantage to conduct such as study as all the included women's PCOS were well defined and diagnosed early

in life and followed into their midlife as they approach the menopause, and their air pollution levels were estimated based on 1-year average exposure for long-term $PM_{2.5}$ assessment. All those allow us to evaluate associations extensively with testing the intermediaries' effect on the association, as well as the interactions. Another strength of our study is that the CHARMIII study included a well-characterized group of women with well diagnosed and defined PCOS cases and controls, providing us the validated data to evaluate the role of PCOS in the association between $PM_{2.5}$ and CAC. This cohort provided well-recorded clinical review, biochemical and radiological data allowing us to evaluate those women' various physiological profiles at their mid-life. Taken together, our findings indicate that women with PCOS could be targeted for prevention of cardiovascular disease and atherosclerosis (CAC), such as making them aware of their potential higher risk of atherosclerosis so that they may change their lifestyle or reduce their environmental exposure as prevention strategies.

4.6 Conclusion

In conclusion, we observed no evidence that $PM_{2.5}$ exposure is associated with the CAC presence or progression among this subset of middle-aged women. However, stronger effect of $PM_{2.5}$ to presence of CAC progression was observed among subgroups of women, such as those ones who were in late peri-menopausal and natural postmenopausal status or without taking CVD medications. Those women may be the more sensitive subgroups impacted by $PM_{2.5}$ to CAC. However, the small sample sizes for each of these subgroup analyses require interpreting these findings with cautions. Future studies with larger sample size and longer follow up should replicate

our analyses. Our work adds to existing evidence on the association of the $PM_{2.5}$ exposure and CAC, with a focusing on the middle-aged women.

4.7 Tables and Figures

Table 4.1 Descriptive statistics at baseline (2001-2003) by PCOS status (N=301), CHARMIII study

Category	Total n, %	PCOS n, %	Controls n, %	Chi-squared p-value
All	301, 100%	136, 45.2%	165, 54.8%	
Current Smoking	48, 15.9%	24, 17.6%	24, 14.5%	0.465
Occasion to drink	228, 75.7%	92, 67.6%	136, 82.4%	0.005
Education: College Degree or above	224, 74.4%	105, 77.2%	119, 72.1%	0.300
White (vs. Black)	252, 83.7%	121, 89%	131, 79.4%	0.025
Current HRT[1] use	46, 15.3%	16, 11.8%	30, 18.2%	0.118
Hypertension	88, 29.2%	45, 33.1%	43, 26.1%	0.182
IFG[2]	61, 20.3%	32, 23.5%	29, 17.6%	0.201
Type 2 Diabetes	23, 7.64%	17, 12.5%	6, 3.64%	0.004
Menopause status				
Pre	191, 63.5%	96, 70.6%	95, 57.6%	
Natural Post	55, 18.3%	20, 14.7%	35, 21.2%	
Surgical Post	48, 15.9%	18, 13.2%	30, 18.2%	0.089
CAC score				
<10	232, 77.1%	90, 66.2%	142, 86.1%	
≥10	69, 22.9%	46, 33.8%	23, 13.9%	<.0001
CAC score				
0	148, 49.2%	51, 37.5%	97, 58.8%	
0<CAC<10	84, 27.9%	39, 28.7%	45, 27.3%	
≥10	69, 22.9%	46, 33.8%	23, 13.9%	<.0001
		[3]**Mean (SD)**		**T test or Wilcoxon-Mann-Whitney test* p-value**
CAC score, median (Q1-Q3)	1.0 (0-7.2)	3.1 (0-16.0)	0 (0-3.4)	<.0001*
$PM_{2.5}$ ($\mu g/m^3$)	16.6(1.3)	16.4(1.4)	16.7 (1.2)	0.094
Age	47.9(5.7)	46.7(5.4)	49 (5.8)	0.001

Table 4.1 Continued

Waist and hip ratio	0.8(0.1)	0.8(0.1)	0.8 (0.1)	<.0001
BMI (kg/m^2)	29.8(6.9)	31.7(7.5)	28.3 (6)	<.0001
Cholesterol, mg/dL	209.2(39.7)	208.3(44.4)	210 (35.5)	0.728
LDLc[4], mg/dL	126.6(35.7)	124.9(40)	127.9 (31.8)	0.467
HDL2[5], mg/dL	18.1(10.5)	17.3(10.2)	18.7 (10.7)	0.243
Triglycerides, mg/dL, Median (Q1-Q3)	114 (77.9-172.0)	126 (82.0-203.0)	101 (77.6-149.0)	0.029*
Glucose, mg/dL, Median (Q1-Q3)	92 (86.0-100.0)	86 (82.0-102.3)	91 (86.8-97.5)	0.202*
Insulin, µU/mL	16.1(10.5)	19.2(12.1)	13.5 (8.1)	<.0001

1. *HRT indicates hormone replacement therapy*
2. *IFG indicates impaired fasting glucose and defined by fasting blood glucose in the range of 110 to 125 mg/dl*
3. *Sample size for PCOS case group is 136 and PCOS control group is 165. Some variables have missing values, and they are: Waist/Hip, BMI, Total cholesterol, HDL2 and Triglycerides had sample sizes for PCOS case and control group: 135 and 164. LDL had sample sizes for PCOS case and control group: 133 and 164*
4. *LDLc standards for low-density lipoprotein cholesterol*
5. *HDL$_2$ stands for high-density lipoprotein cholesterol (HDL) subtraction of HDLt*

Table 4.2 Distributions of cardiovascular risk factors among PCOS cases by CAC presence (CAC<10 vs. ≥10) (N=136), CHARMIII study, 2001- 2003

Category	Level	CAC<10 N, %	CAC≥10 N, %	Chi-squared or Fisher* p-value
	All	90, 66.2%	46, 33.8%	
Current Smoking	No	77, 85.6%	35, 76.1%	
	Yes	13, 14.4%	11, 23.9%	0.171
Occasion to drink	No	27, 30%	15, 32.6%	
	Yes	62, 68.9%	30, 65.2%	0.724
Education Level	High School or lower	23, 25.6%	7, 15.2%	
	College Degree or above	66, 73.3%	39, 84.8%	0.159
Race	White	86, 96%	35, 76%	0.001
	Black	4, 4%	11, 24%	
BMI	<25, Normal	26, 28.9%	4, 8.7%	
	25-29.9, Overweight	25, 27.8%	3, 6.5%	
	≥30, Obese	39, 43.3%	39, 84.8%	<.0001*
Current HRT[1] use	No	81, 90%	39, 84.8%	
	Yes	9, 10%	7, 15.2%	0.372
Hypertension	No	69, 76.7%	22, 47.8%	
	Yes	21, 23.3%	24, 52.2%	0.001
IFG[2]	No	72, 80%	32, 69.6%	
	Yes	18, 20%	14, 30.4%	0.175
Type 2 Diabetes	No	82, 91.1%	37, 80.4%	
	Yes	8, 8.9%	9, 19.6%	0.075
Menopause status	Pre	73, 81.1%	23, 50%	
	Natural Post	9, 10%	11, 24%	0.006
		[3]Mean (SD)		T test or Wilcoxon-Mann-Whitney test^ p-value
$PM_{2.5}$ ($\mu g/m^3$)		16.2 (1.5)	17 (1.2)	0.008
Age		45.9 (4.8)	48 (6.2)	0.027
Waist and hip ratio		0.8 (0.1)	1 (0.1)	0.001
BMI (kg/m^2)		29 (6.5)	37 (6.8)	<.0001
Cholesterol, mg/dL		205.3 (39.8)	215 (52.3)	0.255
LDLc[4], mg/dL		122.5 (34.9)	130 (48.7)	0.316
HDL2[5], mg/dL		18.2 (10.3)	15 (9.7)	0.137
Triglycerides, mg/dL		153.7 (126.7)	194 (228.9)	0.141^
Glucose, mg/dL		93.5 (13.6)	107 (29.3)	0.006^

Table 4.2 Continued

Insulin, µU/mL	16.8 (11.4)	24 (12.2)	0.002

1. *HRT indicates hormone replacement therapy*
2. *IFG indicates impaired fasting glucose and defined by fasting blood glucose in the range of 110 to 125 mg/dl*
3. *Sample size for PCOS case group is 136. Some variables have missing values, and they are: Waist/Hip, BMI, Total cholesterol, HDL2 and Triglycerides had sample sizes for PCOS case: 135. LDL had sample sizes for PCOS case: 133*
4. *LDLc standards for low-density lipoprotein cholesterol*
5. *HDL_2 stands for high-density lipoprotein cholesterol (HDL) subtraction of HDLt*

Table 4.3 Distributions of cardiovascular risk factors for PCOS controls by CAC presence (CAC<10 vs. ≥10) (N=165), CHARMIII study, 2001- 2003

Category	Level	CAC<10 N, %	CAC≥10 N, %	Chi-squared or Fisher* p-value
	All	142, 86.1%	23, 13.9%	
Current Smoking	No	123, 86.6%	18, 78.3%	
	Yes	19, 13.4%	5, 21.7%	0.292
Occasion to drink	No	23, 16.2%	6, 26.1%	
	Yes	119, 83.8%	17, 73.9%	0.248
Education Level	High School or lower	37, 26.1%	8, 34.8%	
	College Degree or above	104, 73.2%	15, 65.2%	0.395
Race	White	118, 83%	13, 57%	
	Black	24, 17%	10, 43%	0.004
BMI	<25, Normal	60, 42.3%	2, 8.7%	
	25-29.9, Overweight	39, 27.5%	6, 26.1%	
	≥30, Obese	43, 30.3%	15, 65.2%	0.001*
Current HRT[1] use	No	115, 81%	19, 82.6%	
	Yes	26, 18.3%	4, 17.4%	1.000*
Hypertension	No	108, 76.1%	14, 60.9%	
	Yes	34, 23.9%	9, 39.1%	0.124
IFG[2]	No	123, 86.6%	13, 56.5%	
	Yes	19, 13.4%	10, 43.5%	0.000
Type 2 Diabetes	No	139, 97.9%	20, 87%	
	Yes	3, 2.1%	3, 13%	0.036*
Menopause status	Pre	83, 58.5%	12, 52.2%	
	Natural Post	28, 19.7%	7, 30.4%	0.292
		[3]**Mean (SD)**		**T test or Wilcoxon-Mann-Whitney test^ p-value**
$PM_{2.5}$ (µg/m^3)		16.7 (1.2)	16 (1)	0.276
Age		49.1 (5.6)	48 (6.7)	0.531
Waist and hip ratio		0.8 (0.1)	1 (0.1)	0.000
BMI (kg/m^2)		27.3 (5.1)	35 (7.2)	<.0001
Cholesterol, mg/dL		210.2 (34.1)	208 (43.6)	0.806
LDLc[4], mg/dL		128.3 (30.6)	126 (39.3)	0.736
HDL2[5], mg/dL		19.4 (10.9)	14 (8.3)	0.014
Triglycerides, mg/dL		120.5 (73.6)	156 (75.3)	0.014^

Table 4.3 Continued

Glucose, mg/dL	92.2 (10.2)	100 (16.4)	0.013^
Insulin, µU/mL	12.1 (5.8)	22 (13.8)	0.001^

1. *HRT indicates hormone replacement therapy*
2. *IFG indicates impaired fasting glucose and defined by fasting blood glucose in the range of 110 to 125 mg/dl Sample size for PCOS case group is 136. Some variables have missing values, and they are: Waist/Hip, BMI, Total cholesterol, HDL2 and Triglycerides had sample sizes for PCOS case: 135*
3. *LDL had sample sizes for PCOS case: 133*
4. *LDLc standards for low-density lipoprotein cholesterol*
5. *HDL_2 stands for high-density lipoprotein cholesterol (HDL) subtraction of HDLt*

Table 4.4 Univariate Logistic regression results of CAC presence and baseline demographic characteristics and cardiovascular risk factors, CHARMIII study: a case-control study (N=301), 2001- 2003

Effect	Odds Ratio	95%	Confidence Interval	
$PM_{2.5}$, $\mu g/m^3$	1.12	0.91	1.37	
PCOS	3.16	1.79	5.56	*
BMI, kg/m^2	1.21	1.15	1.28	*
Age, year	1.01	0.97	1.06	
Education: College Degree or above	1.27	0.67	2.42	
Race	3.19	1.67	6.09	*
Current Smoking	1.89	0.96	3.70	
Occasion to drink	0.62	0.34	1.13	
Current HRT use	1.06	0.51	2.22	
DBP, mm Hg	1.04	1.01	1.08	*
SBP, mm Hg	1.04	1.02	1.06	*
Cholesterol, mg/dL	1.00	1.00	1.01	
LDL, mg/dL	1.00	0.99	1.01	
HDL2, mg/dL	0.96	0.93	0.99	*
Ln (Triglycerides), mg/dL	2.01	1.27	3.19	*
Glucose, mg/dL	1.04	1.02	1.06	*
Insulin, µU/mL	1.08	1.05	1.11	*

Table 4.5 Association between $PM_{2.5}$ (1 µg/m^3) exposure and CAC presence by selected cardiovascular risk factors, CHARMIII study (N=301), 2001- 2003

Effect	N	Odds Ratio	95% Confidence Interval	
$PM_{2.5}$	301	1.12	0.91	1.37
$PM_{2.5}$	301	1.12	0.91	1.38
Age		1.01	0.97	1.06
$PM_{2.5}$	301	1.17	0.95	1.44
PCOS		3.30	1.86	5.84
$PM_{2.5}$	301	1.18	0.96	1.46
Age		1.04	0.99	1.09
PCOS		3.63	2.01	6.56
$PM_{2.5}$	300	1.17	0.91	1.50
Age		1.06	1.00	1.13
PCOS		2.31	1.16	4.56
BMI		1.21	1.14	1.27
$PM_{2.5}$	298	1.16	0.91	1.49
Age		1.06	1.00	1.13
PCOS		2.26	1.14	4.48
BMI		1.20	1.14	1.27
HDL_2		0.99	0.96	1.03
$PM_{2.5}$	300	1.18	0.92	1.52
Age		1.05	0.99	1.12
PCOS		2.31	1.17	4.59
BMI		1.20	1.14	1.27
SBP		1.01	0.99	1.04
$PM_{2.5}$	300	1.17	0.91	1.49
Age		1.06	1.00	1.13
PCOS		2.30	1.16	4.56
BMI		1.21	1.15	1.28
DBP		0.99	0.95	1.04
$PM_{2.5}$	298	1.16	0.91	1.49
Age		1.06	1.00	1.13
PCOS		2.21	1.10	4.42
BMI		1.20	1.14	1.27
Ln (Triglycerides)		1.15	0.64	2.06
$PM_{2.5}$	298	1.16	0.90	1.49
Age		1.07	1.01	1.13
PCOS		2.07	1.03	4.17
BMI		1.18	1.11	1.25
Insulin		1.03	1.00	1.06

Table 4.5 Continued

$PM_{2.5}$	298	1.14	0.89	1.47
Age		1.05	0.99	1.12
PCOS		2.09	1.05	4.17
BMI		1.19	1.13	1.26
Glucose		1.02	1.00	1.04
$PM_{2.5}$	244	1.11	0.82	1.49
Age		0.98	0.89	1.08
PCOS		1.36	0.59	3.14
BMI		1.22	1.13	1.31
Insulin		1.03	0.99	1.08
Menopausal status		4.50	1.38	14.72
SBP		1.03	1.00	1.06
Smoking status		3.20	1.22	8.38
$PM_{2.5}$	244	1.05	0.77	1.43
Age		1.00	0.91	1.09
PCOS		1.99	0.83	4.80
Race		5.25	1.94	14.24
Menopausal status		4.77	1.49	15.26
BMI		1.20	1.11	1.29
Insulin		1.03	0.99	1.07

Table 4.6 Association between $PM_{2.5}$ (1 µg/m^3) exposure and CAC presence among PCOS cases (N=136), CHARMIII study, 2001- 2003

Effect	N	Odds Ratio	95% Confidence Interval	
$PM_{2.5}$	136	1.42	1.07	1.87
$PM_{2.5}$	136	1.44	1.08	1.92
Age		1.09	1.02	1.17
$PM_{2.5}$	135	1.51	1.12	2.05
Age		1.10	1.02	1.18
Education		2.66	0.97	7.26
$PM_{2.5}$	135	1.44	1.02	2.04
Age		1.11	1.02	1.21
BMI		1.20	1.11	1.29
$PM_{2.5}$	134	1.44	1.02	2.05
Age		1.12	1.03	1.22
BMI		1.20	1.11	1.30
HDL_2		1.01	0.96	1.07
$PM_{2.5}$	135	1.50	1.05	2.15
Age		1.10	1.01	1.20
BMI		1.18	1.10	1.27
SBP		1.03	0.99	1.07
$PM_{2.5}$	134	1.42	1.00	2.02
Age		1.13	1.03	1.23
BMI		1.18	1.09	1.27
Insulin		1.02	0.99	1.06

Table 4.7 Association between $PM_{2.5}$ (1 µg/m^3) exposure and CAC presence among women without PCOS (N=164), CHARMIII study, 2001- 2003

Effect	N	Odds Ratio	95% Confidence Interval	
$PM_{2.5}$	165	0.82	0.57	1.17
$PM_{2.5}$	165	0.80	0.55	1.15
Age		0.97	0.89	1.05
$PM_{2.5}$	164	0.81	0.56	1.17
Age		0.96	0.88	1.04
Education		0.62	0.23	1.66
$PM_{2.5}$	165	0.84	0.56	1.25
Age		1.00	0.91	1.09
BMI		1.21	1.12	1.32
$PM_{2.5}$	164	0.84	0.56	1.26
Age		1.00	0.92	1.10
BMI		1.20	1.11	1.31
HDL_2		0.97	0.91	1.03
$PM_{2.5}$	165	0.85	0.57	1.26
Age		1.00	0.91	1.09
BMI		1.21	1.11	1.32
SBP		1.01	0.97	1.04
$PM_{2.5}$	164	0.84	0.56	1.26
Age		1.00	0.91	1.09
BMI		1.15	1.03	1.28
Insulin		1.05	0.98	1.13

Table 4.8 Effect modification of PCOS status on the association between $PM_{2.5}$ and CAC presence, CHARMIII study, 2001- 2003

Stratum in the interaction models	Total Log Odds	Total OR	p for interaction
Unadjusted model*			
Reference	0.0000	1.0000	
Effect of total effect of $PM_{2.5}$, PCOS, and interaction term of $PM_{2.5}$ and PCOS in the interaction model	-7.5813	0.0005	0.018
Effect of $PM_{2.5}$ in the interaction model	-0.2013	0.8177	
Effect of PCOS in the interaction model	-7.9290	0.0004	
Model adjusted for Age^			
Reference	0.0000	1.0000	
Effect of total effect of $PM_{2.5}$, PCOS, and interaction term of $PM_{2.5}$ and PCOS in the interaction model	-7.1566	0.0008	0.024
Effect of $PM_{2.5}$ in the interaction model	-0.1774	0.8375	
Effect of PCOS in the interaction model	-7.5081	0.0005	
Model adjusted for Age, race, BMI, menopausal status#			
Reference	0.0000	1.0000	
Effect of total effect of $PM_{2.5}$, PCOS, and interaction term of $PM_{2.5}$ and PCOS in the interaction model	-10.2939	0.00003	0.037
Effect of $PM_{2.5}$ in the interaction model	-0.3486	0.70568	
Effect of PCOS in the interaction model	-10.6335	0.00002	

**For unadjusted model, $OR_{11}/(OR_{10}*OR_{01})=0.0005/(0.0004*0.8177)=1.53>1$, therefore there is a positive multiplicative interaction*

^For model adjusted for age, $OR_{11}/(OR_{10}*OR_{01})=0.0008/(0.0005*0.8375)=1.91>1$, therefore there is a positive multiplicative interaction

#For model adjusted for age, BMI, race and menopausal status, $OR_{11}/(OR_{10}*OR_{01})=0.00003/(0.00002*0.70568)=1.53>2.13$, therefore there is a positive multiplicative interaction.

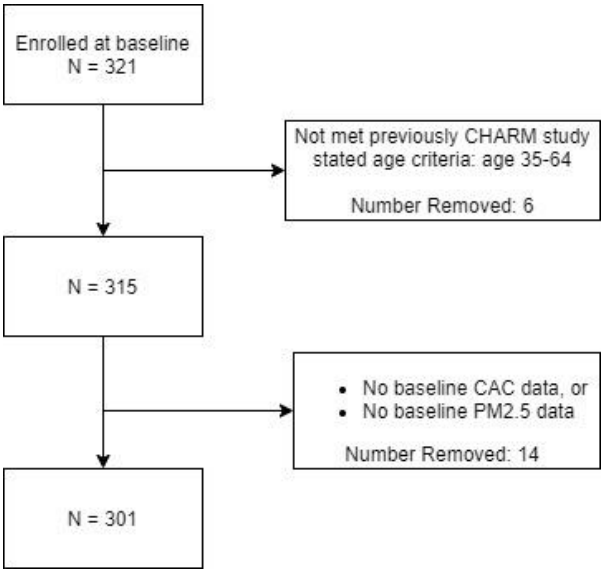

Figure 4.1 Data reduction for the participants included in the baseline and final analyses, CHARMIII study, 2001-2003

5.0 Association of Exposure to Particulate Matter ($PM_{2.5}$) Air Pollution and Coronary Artery Calcification in Middle-Aged Women Transitioning Through Menopause: The Study of Women's Health Across the Nation (SWAN)

5.1 Abstract

Objective: To evaluate the association of $PM_{2.5}$ ambient air pollution and coronary artery calcification in middle-aged women, and to study the role of the menopausal transition in this association.

Methods: We evaluated 366 women from the SWAN Heart Ancillary study (Study of Women's Health across the Nation). Women who had data on atherosclerosis measured by the CAC Agatston score, menopause profiles' measures, and baseline $PM_{2.5}$ exposure were included in our analyses. Logistic regression models were used to estimate the cross-sectional association between $PM_{2.5}$ exposure and CAC presence (categorized into: CAC Agatston score <10 vs. ≥10) and CAC progression, adjusting for prior selected covariates. CAC progression was defined as present if 1) baseline CAC=0 and follow-up CAC>0, or 2) baseline CAC>0 to <100 and annualized change in CAC≥10, or 3) baseline CAC≥100 and annualized percent change in CAC≥10%. The follow-up CAC scans were conducted in 69% of the participants (N=253). Generalized linear mixed models were used to evaluate the association between $PM_{2.5}$ and repeated measures of detectable CAC (categorized into: CAC Agatston score=0 vs. >0), adjusting for prior selected time-varying covariates. Potential modifiers, time-varing menopausal status and baseline $PM_{2.5}$ exposure time to final menstral period (FMP), were tested by adding those terms with $PM_{2.5}$ as interaction terms into the final model.

Results: The study included 366 women aged 51.3 years (N=198 pre- or early perimenopausal, N=129 late perimenopausal or natural postmenopausal, and N=39 unknown due to hysterectomy or hormone therapy use). At baseline visit, there were 289 (79%) women with CAC less than 10; and 77 (21%) women with CAC greater or equal to 10. Annual mean $PM_{2.5}$ levels were 16.5 ± 1.3, and 16.6 ± 1.4 $\mu g/m^3$ respectively, among women with and without presence of CAC (p=0.656). Out of those with two time points of CAC measures (N=253), there were 200 women (79.1%) without presence of CAC progression, while 53 women (20.9%) had presence of progression. Annual mean $PM_{2.5}$ levels were 16.6 ± 1.0, and 16.7 ± 1.7 $\mu g/m^3$ respectively, among women with and without presence of CAC progression (p=0.538). Among the subset with observed FMP, a total of 27.6% of women's estimated $PM_{2.5}$ exposure date was 2 years prior to FMP; 30.6% were within 2 years to FMP, and 11.7% of them had exposure measured 2 years after FMP. There was no association between $PM_{2.5}$ and CAC presence or progression, in both cross-sectional and longitudinal analyses. All the tested interactions were not statistically significant.

Conclusions: Overall, there was no association between $PM_{2.5}$ exposure and CAC among this cohort of midlife women. Menopausal transition did not have a significant effect modification on the association of $PM_{2.5}$ and CAC presence or progression in these middle-aged women. Replication of this work in other groups of mid-life women with larger sample size would be needed to confirm our findings.

Key Words: $PM_{2.5}$, air pollution, subclinical atherosclerosis, coronary artery calcification, menopausal transition, final menstrual period, middle-aged women

5.2 Introduction

Air pollution is associated with increased risk in cardiovascular disease (CVD), the leading cause of mortality in the US (1). Fine particulate matter (particles ≤ 2.5μm in aerodynamic diameter [$PM_{2.5}$]) is a major component of air pollution known to affect cardiovascular health in the general population (22). The most commonly known biological mechanism behind the association of $PM_{2.5}$ and cardiovascular health is that inhalational exposure to $PM_{2.5}$ triggers inflammation and generates reactive oxygen species release, which leads to endothelial dysfunction and detrimental effects on vascular cells (99).

Atherosclerosis, a pre-clinical stage of CVD, is commonly measured by CT scan using coronary artery calcification (CAC) Agatston score (63). CAC highly correlates with the degree of atherosclerosis and has been shown to be predictive of future CVD risk (160). CAC has been shown to be a better marker for CVD risk prediction than other measures, including carotid plaque and intima-media thickness (IMT) (58), as the presence of CAC well predicts incident CVD especially among middle-aged adults without pre-existing CVD (7,59).

Observational studies reported equivocal evidence on associations between $PM_{2.5}$ and CAC (8–14). Most studies reported no association, but two studies conducted more recently found that long-term $PM_{2.5}$ exposure increases CAC risk, with reporting an even stronger effect of $PM_{2.5}$ on CAC among postmenopausal women (10,14). Those include one recent study by Wang et al, which reported a positive association using a Chinese cohort (N=1732 out of 3790 women were postmenopausal women); further in the subgroup analysis they found that $PM_{2.5}$ exposure had an even stronger effect on CAC measures among postmenopausal women (larger effect size),

compared to the general population (14). Another study by Kaufman et al., 2016 assessed the associations of progression of CAC and exposure to $PM_{2.5}$ using the US Multi-Ethnic Study of Atherosclerosis (MESA) cohort (10). They also reported that there was a positive association between $PM_{2.5}$ exposure and CAC progression. Additionally the association between $PM_{2.5}$ and CAC progression was stronger among women than men (10). These findings were consistent with previous observational studies reporting that $PM_{2.5}$ increases risk of women's cardiovascular health and CVD risk in middle-aged and elderly populations (37,95,130). As CAC is one of the strongest CVD risk prediction tools and correlates well with the overall burden of atherosclerosis, studying the association between $PM_{2.5}$ and outcome measure of CAC other than CVD provides opportunity for reducing CVD burden (e.g. by reducing $PM_{2.5}$ exposure if there is an association between $PM_{2.5}$ and CAC). Additionally, previous studies suggest midlife women are particularly vulnerable to atherosclerosis risk as they transition through menopause (72,180,181). They undergo multiple adverse physiological alterations that could increase their risk for CVD. Thus, evaluating associations between $PM_{2.5}$ and CAC in midlife women would be critical to better understand the contribution of the menopausal transition to this association.

In the Study of Women's Health across the Nation (SWAN) study, menopause status has been carefully classified based on women's self-reported bleeding history (204). Menopause status was classified as premenopausal (Pre; menses in the last 3 months with no irregularity), early-perimenopausal (EP; menses in the last 3 months with irregularity), late- perimenopausal (LP; no menses for at least 3 months, but less than 12 months) and postmenopausal (Post; no menses for at least 12 months). Women with surgical menopausal, underdetermined menopausal status due to hormone therapy (HT) use, or no menopausal status information available were excluded from the analyses focusing on effect modification by menopause status.

The one to two years around FMP has proven to be a critical period of the menopausal transition when multiple metabolic and various physiological changes (e.g. lipids, hormonal changes) occur (120). Whether those changes occuring during this period compound the harmful effect of $PM_{2.5}$ to the cardiovascular health (e.g. atherosclerosis measured by CAC) remains unknown. Therefore, in addition to considering the menopausal status as a measure of menopausal transition, the estimated $PM_{2.5}$ exposure time to FMP can be another way to evaluate the role of menopausal transition and its effect on the association between $PM_{2.5}$ and CAC. Hence, to fill in the research gap, we conducted the current study to examine the association between $PM_{2.5}$ and CAC (presence and progression) in middle-aged women, and to evaluate whether menopausal transition (by using menopausal status and exposure time to FMP) modifies this association. The exposure measure time in the SWAN heart cohort is very close to the CAC baseline measure time. By anchoring measurement time to FMP, we can explore whether closer to FMP added to the harmful effect of $PM_{2.5}$ on CAC risk.

There has been little attempt to evaluate the association between long-term exposure to $PM_{2.5}$ and CAC progression over time. Previous studies found that CAC progression potentially adds further predictive value and closely correlates with CVD risk in low-risk populations (56,205). Studying the impact of $PM_{2.5}$ on CAC progression during menopausal transition can provide a unique opportunity to better capture the dynamic changes occurring during the menopausal transition and to see whether those plus the effect of $PM_{2.5}$ increase the risk of CAC progression.

The menopausal transition period, therefore, could be viewed as a critical period to capture long-term chronic effects of $PM_{2.5}$ on CAC that otherwise might not be detectable. In addtion, the previous studies reported that $PM_{2.5}$ exposure is associated with the increased risk of CAC among

postmenopausal women (higher risk among postmenopausal women vs. general population) (14). This implies that the stronger effect of $PM_{2.5}$ exposure has on CAC risk may be due to the effects of aging or the effects of menopause.

Our objective was to investigate the association between $PM_{2.5}$ and CAC presence and progression both cross-sectionally and longitudinally among middle-aged women, and to evaluate whether the menopause transition modifies this association using the Study of Women's Health Across the Nation (SWAN) Heart study. We hypothesized that exposure to $PM_{2.5}$ is associated with increased CAC presence and progression among middle-aged women and that the harmful effect of $PM_{2.5}$ may be stronger in late-perimenopause and natural postmenopausal women or when the measures closer to the FMP because that signifies a period of dynamic physiologic changes.

5.3 Methods

Study Participants

The SWAN study is a community-based multi-site longitudinal, epidemiologic study designed to examine the physical, biological, psychological and social changes during the menopause transition (206). Briefly, the SWAN study was conducted at seven study sites across the U.S. (Boston, MA; Detroit, MI; Oakland, CA; Los Angeles, CA; Pittsburgh, PA; Chicago, IL; and Newark, NJ), and 3302 women aged 42 to 52 years were recruited during the period 1996-1997. The eligibility criteria for the SWAN study were women who had an intact uterus, ≥1 ovary, ≥1mestrual period within the past 3 months, and were not hormone therapy user within the past 3 months. The SWAN Heart is an ancillary study to SWAN designed to assess subclinical atherosclerosis measures in women at midlife within two SWAN clinical sites: Pittsburgh and

Chicago. Baseline CAC measurements were obtained in participants attending SWAN visits 4 - 7 (2001 - 2004). These participants were invited back to obtain a 2-year follow-up measure at a subsequent visit corresponding to SWAN visits 6 - 9 (2002 - 2006). By design, the two SWAN Heart study sites recruited White and Black women.

All participants of the SWAN Heart study who had Electron Beam Tomography (EBCT) scans available at the SWAN Heart baseline or follow-up visit were considered to be included in the study. Of 608 SWAN Heart participants, 586 had CAC scans at either SWAN Heart baseline visit (2001-2004) or follow-up visit (2002-2006). For the current analyses, women were excluded if they had no $PM_{2.5}$ baseline data by design (women from Chicago WISH study site, N=183). In addition, we excluded women who reported CVD during the SWAN Heart follow-up period (n=11) or had $PM_{2.5}$ data and menopausal status data missing (n=26) (Figure 5.1). The final analytic sample included 366 women for cross-sectional analyses of association between $PM_{2.5}$ and CAC presence. For cross-sectional analyses of association between $PM_{2.5}$ and CAC progression, 253 women who have both SWAN Heart baseline and follow-up CAC data available were included. For the longitudinal association of $PM_{2.5}$ with CAC presence over time (repeated measures of CAC), 336 women with 619 observations with each woman having 1 or 2 measurements time points were included.

Figures 5.1 shows in more details of the data reduction of our analyzed data for the baseline CAC set and complete CAC (both baseline and follow-up CAC) set. Additionally, we excluded 110 women for whom FMP date was not observed (Figure 5.2) for the FMP related interaction tests. For the interaction with menopausal status, we excluded 39 women with surgical menopause or had undetermined menopausal status due to HT use, as the effect of $PM_{2.5}$ to CAC among those women could be different, which are not our study of interest. This resulted in an analytical sample

size of at most 327 for this analysis if there are no other covariates missing in the model. The Institutional Review Board of both Pittsburgh and Chicago sites approved this study. Written informed consents were obtained for all the participants.

Exposure to $PM_{2.5}$ assessment

The air pollution measurements were concurrent with the SWAN Heart study baseline measures and partially overlap with the follow-up measures of the outcomes. The estimated annual mean air pollution data at SWAN Heart baseline visit were available to use for each of our included participants in this study. For each participant, an annualized mean exposure to $PM_{2.5}$ was calculated for a one-year exposure period prior to each of the SWAN Heart baseline and follow-up visit. The assessment for $PM_{2.5}$ exposure is a standard exposure computation method, and the detailed exposure assessment and the address geo-coding are detailed elsewhere (88). Briefly, residential addresses of participants were collected and geo-coded. Daily $PM_{2.5}$ were retrieved from the AQS DataMart (162). Exposure to $PM_{2.5}$ was determined by one monitor if it was located within 20 km from the participant's address, and were collected for a one-year exposure period prior for each participant. Annual exposure to these pollutants was defined as 360 days before the study visit (88). If the participant moved during the year prior to her visit, both locations were considered when assigning exposure (88). The corresponding annual mean exposure of $PM_{2.5}$ before the SWAN Heart baseline and follow-up visits were available and extracted for the analyses. Levels of $PM_{2.5}$ at both sites, for baseline visit and follow-up were highly correlated each other. As all exposure data were available at SWAN heart baseline visit, but plenty of follow-up visits' exposure data were missing, we used baseline $PM_{2.5}$ data as exposure for both visits for the analyses.

Coronary artery calcium assessment

CAC was generated using the C-150 Ultrafast CT Scanner (GE Imatron). An initial scout scan was performed to identify anatomic landmarks. To evaluate the coronary arteries, 30 to 40 contiguous 3-mm-thick transverse images were obtained from the level of the aortic root to the apex of the heart during maximal breath holding. All scan data were saved to an optical disk for central scoring, using a Base Value Region of Interest (BVROI) computer program (Accu-Image). This software program implements the Agatston scoring method (63). CAC was defined as a hyperattenuating lesion >130 Hounsfield units, with an area of at least 3 pixels. A calcium score was then calculated for each region of interest by multiplying the area of all significant pixels by a grade number 1,2,3 or 4 indicatives of the peak CT number (Houndsfield units) (Agatston scoring method) (63). The individual region-of-interest scores were then summed for a total calcification score. The scoring system had high reproducibility as measured in 40 older adults selected to have a wide range of disease. The intraclass correlation for CAC scores was 0.99 (62).

Blood Assays

A fasting blood sample was obtained and analyzed with standardized protocols at both the baseline and follow-up visits of the SWAN Heart ancillary study. Lipids (total cholesterol, HDL, LDL, and triglycerides), glucose, and insulin were measured at the Medical Research Laboratories (Lexington, KY). Total cholesterol and triglyceride levels were analyzed using enzymatic methods on a Hitachi 747 analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN) (207), and high-density lipoprotein cholesterol was isolated using heparin-manganese (208). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation (202). Serum insulin was measured by a radioimmunoassay (DPC Coat-a-count) and glucose was measured with a hexokinase-coupled reaction (Boehringer Mannheim Diagnostics). The homeostasis model

assessment (HOMA) index was calculated from fasting insulin and glucose as follows: (insulin [in uIU/ml x glucose [in mmol/L]/22.5 (202,209).

Inflammatory/hemostatic markers examined in this study included high-sensitivity C-reactive protein (hs-CRP), tissue-type plasminogen activator (tPA), and plasminogen activator inhibitor Type 1 (PAI-1). C-reactive protein (CRP) was measured in serum or plasma by immunonephelometry using Behring reagents on the Behring Nephelometer II. Polystyrene particles coated with antibodies to CRP was agglutinated when mixed with samples containing CRP. The intensity of the light scattered in the nephelometer is proportional to the concentration of CRP in the sample. Results was evaluated by comparison with a standard of known concentration. Coefficient of variation is approximately 5%. tPA is measured in plasma by ELISA, enzyme linked immunoassay using American Diagnostica Imubind reagent (210). Coefficient of variation is approximately 8%. PAI-1 was measured using a solid phased monoclonal antibody and a second enzyme-labeled goat antiserum for detection (IMUBIND plasma PAI-1 enzyme-linked immunosorbent assay; American Diagnostica, Greenwich, Connecticut). PAI-1 monthly interassay CVs were 5% to 9% and 4% to 9% at mean concentrations of 7 and 22.5 ng/dL, respectively. A double antibody in an enzyme-linked immunosorbent assay (American Diagnostica) measured t-PA-ag, with a human single chain t-PA-ag as a standard calibrated against an international standard (National Institute for Biological Standards and Control, Hertfordshire, United Kingdom). Monthly interassay CVs were 4.7% to 8.7% and 3.8% to 7.8% at mean concentrations of 5.6 and 11 ng/dL, respectively.

Endogenous sex hormones were measured at the University of Michigan Endocrine Laboratory using the Automated Chemilumisence System -180 automated analyzer (Bayer Diagnostics Corp., Norwood, MA). Estradiol (E2) was measured using a modified, off-line

Automated Chemilumisence System: 180 (E2-6). The lower limit of detection (LLD) was between 1 and 7 pg/mL. The E2 assay modifies the rabbit anti-E2-6 ACS-180 immunoassay to increase sensitivity, with a lower limit of detection (LLD) of 1.0 pg/mL. The inter- and intra-assay coefficients of variation averaged were 10.6% and 6.4%, respectively. Follicle-stimulating hormone (FSH) was measured by a modification of a manual assay kit (Bayer Diagnostics) utilizing two monoclonal antibodies directed to different regions on the beta subunit, with an LLD of 1.05 mIU/mL. The inter-and intra-assay coefficients of variation were 11.4% and 3.8%, respectively. Since estradiol and FSH show dynamic changes throughout the menstrual cycle, cycle-day of blood draw (within 2-5 days of the menstrual cycle or not) was considered when adjusting for these hormones.

Other study variables

Risk factors' measures and demographic characteristics data of those included women were also collected, including information on anthropometrics, CVD risk factors (e.g. blood pressure, lipids, insulin levels), and menopausal transition related variables. Race/ethnicity, income (grouped into more than $75,000 family annual income vs. less), and education attainment (coded into less than college vs. some college vs. post college degree) were determined from the SWAN screening interview. Age, smoking status (current versus past/never), drinking history (any drinking, Yes vs. No), chronic health morbidity, medications' use, physical activity were derived from questionnaires and interviews administered during annual visits, including the visit concurrent with the CAC measures. As physical activity scores were available only at SWAN visit 3, 5 and 6, we assigned the values to visit 4 and 7 with the available value from the visit before or after that visit, and included it in the model, and this has been done by previous SWAN study (211). Medication use was defined as yes if the participant reported use of medications for

hypertension, diabetes mellitus, or high cholesterol (based on ten-item list of prescription drugs). Morbidity was defined as yes if the participant reported history of hypertension, or diabetes mellitus. Height and weight were measured at all visit and BMI was calculated (kg/m^2). Blood pressure was the average of two seated measurements.

Effect modifier variables: Menopausal status and $PM_{2.5}$ exposure time to FMP

Menopausal status was determined based on frequency and regularity of menstrual bleeding as follows: (1) premenopause, no perceived change in bleeding; (2) early perimenopause, perceived change in cycle interval but at least 1 menstrual period within the past 3 months; (3) late perimenopause, 3 consecutive months of amenorrhea; (4) postmenopause,12 consecutive months of amenorrhea. Women who had surgical menopause or underdetermined menopausal status due to HT use were excluded from the analyses related to the menopause status in our study. In addition, because the sample size of premenopausal women was small and their characteristics and E2 and CAC levels were similar to those of early perimenopausal women (data not shown), premenopausal and early perimenopausal women were combined in one group in the current study (77). Similarly, the late perimesnopausal and postmenopausal women were combined into one group for analyses in the current study. In longitudinal analyses, menopausal status at both SWAN Heart baseline and follow-up CAC measures' visits were categorized into: pre-/ early peri- group and late peri-/ natural post-menopausal group. In addition, ever used hormone therapy at both SWAN Heart baseline and follow-up CAC measures' visits were adjusted for in longitudinal analyses.

Based on menstrual bleeding patterns and reproductive hormones, as well as monthly menstrual calendars, precise identification of the timing of the FMP was estimated for the women of SWAN heart study. The FMP observed data was available to use for half of the participants in

SWAN heart study. For women who did not have the observed FMP data available, the imputed FMP data were available to use. The algorithm applied to impute FMP is provided in the appendix material. In the sensitivity analyses, we reran the final model of interaction of $PM_{2.5}$ and time to FMP on association between $PM_{2.5}$ and CAC to women with imputed and observed FMP and compared the results among women with observed FMP. $PM_{2.5}$ exposure time to FMP were calculated as the estimated $PM_{2.5}$ measure date (blood draw date) minus date of observed FMP, and we checked the dates variables, the blood draw data is very close to the date of CAC SWAN heart baseline scan date in our included cohort. These created variables were then used to generate flags for marking those exposures measured 1 or 2 years prior FMP, within 1-2 years of FMP, and 1 or 2 years after FMP. The exposure time since FMP, and the created FMP categorical variables described were used for our analyses related to evaluating the effect modifications between $PM_{2.5}$ and time to FMP. As the categorical time to FMP cannot be created using the imputed and observed FMP dataset, we conducted the sensitivity analyses for the interaction tests related to FMP using the exposure time since FMP among women with imputed and observed FMP.

Statistical analyses

The distribution of CAC was markedly skewed and could not be normalized using traditional mathematical transformations. Hence, as described below, CAC was categorized into a binary variable for cross-sectional analyses and we used logistic regression modelling. We categorized CAC at both visits as either 0 or 1 (CAC<10 vs.CAC≥10), and applied a generalized linear mixed model with random intercept to assess repeated measures of CAC>0, adjusting for prior selected covariates. More specifically, CAC Agatston scores were categorized into binary variables of presence of CAC or not: 1) CAC<10; 2) CAC≥10, for cross-sectional analyses. These cutoffs of presence of CAC have been widely used in the previous study (70). In addition, to

maximize clinical relevance, the definitions of detectable CAC cut-points used in clinical practice and widely cited in the literature (71), alternative approach to categorize it into binary variable as detectable CAC or not, 1) CAC=0; and 2) CAC>0, were also used for cross-sectional analyses. Those results of detectable CAC were included in the appendix materials and the results of CAC presence were presented as main findings. Because the presence of CAC (CAC≥10) group of women are the ones having more confirmed CAC presence than the ones with CAC>0, which is also more commonly used cutoff in the study of low-risk subjects, including the previous SWAN heart study (77).

For repeated measure of CAC, we coded CAC scores at baseline and follow-up visits into detectable CAC (1) CAC=0; (2) CAC>0. The reason we did not use the cutoff as the above-mentioned CAC presence (CAC<10 vs. ≥10) is that the SWAN heart follow-up CAC were not conducted among all participants, and only 65 women had CAC≥10 (compared with N=121 women had CAC>0, at follow-up visit). Considering the interaction tests which we had even samller number of women in each category of the menopausal status and time to FMP, detectable CAC for our longitutinal analysis provided more power to build the stable models with the larger sample size in each category. We then included the repeated measures of CAC into the generalized linear mixed models to evaluate the association between $PM_{2.5}$ and repeated measures of CAC (results were included in the appendix materials). While evaluating the association longitudinally, all the covariates which had repeated measures available were included in the models as time-varying variables, such as menopausal status, HDL-C, Ln (CRP), and Ln (HOMA).

CAC progression was evaluated as presence of CAC progression, and a dichotomous variable of significant progression was created. The present of CAC progression was defined as: 1) CAC score was >0 at follow-up if baseline CAC= 0, or 2) annualized change in CAC score was

≥10 if baseline CAC score >0 to <100, or 3) annualized percent change in CAC score was ≥10% if baseline CAC score ≥100 (76). The detailed description of this definition has been previously published before and was used in a study of low-risk subjects (212).

For interaction tests, both $PM_{2.5}$ and time since FMP and flags of categorical time to FMP were created for the analyses. Since one to two years around FMP has proven to be a critical period in women's lives as the dynamic metabolic and various physiological changes occur (120), we created the flags for $PM_{2.5}$ exposure time since FMP using the cutoff points: 1) 1 year before/within/after FMP; and 2) 2 years before/within/after FMP. We looked at the distribution of our outcomes of interests using each of the cutoffs to determine that the optimal cutoff to use is 2 years around FMP, as this categorization provided larger sample size for each subgroups and giving more power to detect the potential association, compared to the cutoff of 1 year. Therefore, all the results related to cutoff of 2 years were selected to present as main findings (cutoff of 1-year findings can be found in the appendix materials). More specifically, we created three categories using each cutoff point for the $PM_{2.5}$ exposure time to FMP. For the cutoff of 2 years: "pre-": >2 years before FMP; "middle": within 2 years before and after FMP; "post-": >2 years after FMP. Models including the interaction terms were build using both cutoff (1 year and 2 years) of categorical time to FMP and time since FMP, and the results of the cutoff of 2-year were presented as main findings, while other results were included in the appendix materials. All of the analyses related to evaluating FMP as effect modifier on the association between $PM_{2.5}$ and CAC were conducted using the observed FMP data available and those results are presented as main findings. Similar analyses including all women with both observed and imputed FMP were conducted as sensitivity analyses (results were included in the appendix materials). To account for the uncertainty in the imputation process, our final estimates incorporated within and between

imputation variability as recommended by Little and Rubin, 2019 (213,214). The utilized approach essentially taking a sample of size 10 from a woman estimated distribution of FMP date, and incorporating that distribution's variability (imputation uncertainty) in analyses accordingly, see imputation algorithm in appendix.

Descriptive analyses were performed for the overall population, and by categorical CAC. Transformation of data for the non-normally distributed triglycerides, E2, HOMA, t-PA, PAI-1, and CRP were used, according to the previous SWAN study. Bivariate analyses that were conducted include: 1) T tests or Wilcoxon-Mann-Whitney tests for all continuous variables, by CAC (<10 vs. ≥10), depending on whether the data were normally distributed or not; 2) Chi-square tests or Fisher's exact tests for all categorical variables, by CAC presence (<10 vs. ≥10). Additionally, univariate analyses were conducted for all the potential covariates by outcome detectable CAC (0 vs. >0). All bivariate analyses were also conducted for baseline detectable CAC (0 vs. >0), and the results were included in the appendix materials. Univariate analyses were conducted for all the potential covariates by outcome of presence CAC (<10 vs. ≥10). Additionally, for CAC progression, the univariate analyses were conducted for all the potential covariates by outcome: presence of CAC progression (Yes vs. No). What's more, we have also presented the univariates analyses results of the generalized linear mixed model for repeated measures of CAC and those time-varying and time invariant risk factors, and this helped with candidate covariates' selection of the repeated measures analyses.

For the model building, logistic regression models were used for binary CAC (CAC presence; detectable CAC; and presence of CAC progression) to assess the association between $PM_{2.5}$ and CAC presence and progression cross-sectionally. Generalized linear mixed models were used for repeated measures of CAC to evaluate the association between $PM_{2.5}$ and repeated

measures of CAC. The statistical covariates selections strategy include: 1) Covariates that were found to be significantly associated with study outcomes were considered as potential covariates; 2) Independent variables (showing statistically significant: $p<0.05$) in the univariate analyses were considered as potential covariates; 3) Variables were removed using model selection (stepwise selection models), and those covariates with a p threshold of >0.2 were removed from the models (the removals did not change the effect size >10% and significance); 4) Correlated covariates can't be both included into the same model, as it could lead to collinearity issues (e.g. BMI and obesity; menopausal status and time to FMP; total cholesterol and HDL/LDL; SBP and DBP). What's more important, those chosen study covariates were based on a priori knowledge (from the literature reviews) of risk factors of CAC (e.g. SBP, BMI, LDL cholesterol, and HOMA). The additionally adjusted for those factors above can help with determine whether those factors explain any detected changes in CAC. Those selected models, which presented in our paper, were unadjusted; minimally adjusted (race, age, income, site), adjusted for CVD risk factors; adjusted additionally for menopausal status and HT use models; and final parsimonious model (by removing variables using stepwise selection methods). It is worthy to note that those included covariates were not the same in those different outcome assessment models for CAC presence, detectable CAC, presence of CAC progression, and repeated CAC measures overtime. The selected candidate covariates for each of those outcomes depended on their univariate or bivariate analyses' results.

Effect modifications by menopausal status and by exposure time to FMP were tested in the final model; and the corresponding stratified analyses in this model were presented to provide estimates of the association between $PM_{2.5}$ and CAC in each group separately. Additionally, in order to identify high risk population, exploratory subgroup analyses by BMI, CRP levels, race, smoking status, age, total cholesterol, LDL, HDL, were conducted for each of those above-

mentioned outcomes and the results were included in the appendix tables. Furthermore, sensitivity analyses were conducted using the final models among women without history of other diseases (hypertension and diabetes mellitus), without taking medications (cholesterol, blood pressure, or diabetes mellitus), never had hormone use, and with imputed and observed FMP (appendix tables).

All statistical analyses were performed using SAS 9.4 (Cary, North Carolina). Two-sided p-values≤0.05 were considered statistically significant.

5.4 Results

Overall, the study included 366 women who participated in the SWAN Heart study and 253 who had follow-up visits of CAC scans. At baseline visit, there were 289 (79%) women with CAC less than 10; and 77 women (21%) with CAC greater or equal than 10. In addition, at baseline visit, there were 194 women (53%) with CAC equal to 0; and 172 women (47%) with CAC greater than 0. Out of those with two time points of CAC measures (N=253), there were 200 (79.1%) women without presence of CAC progression, while 53 (20.9%) women had presence of CAC progression. (Table 5.1) Presence of CAC progression by baseline CAC is presented in the Figure 5.3. A total of 256 women had observed FMP, and the interactions tests related to FMP were conducted including those participants. There were 253 women with menopausal status data, and 39 women with unknown data due to hysterectomy or hormone therapy use were excluded from the analyses related to menopause status. The detailed data reduction figures are presented in Figure 5.1 and Figure 5.2.

Out of those with baseline CAC measures (N=366), 35.2% percent of women were in late peri-menopausal or natural menopause, and 54.1% of them were in pre- and early peri-menopausal

status. (Table 5.1) At follow-up visit (~2 years later), women's mean age was 53.1 ± 2.7 years, and among those women with menopausal status data (N=226), 69% were in late peri-menopausal or natural menopausal status (data not shown). The mean age was 51.3 (SD=2.8) years old at SWAN heart baseline visit, 65% of 366 included women were Caucasian, and 84% had a college degree or above. The mean BMI was 29 ± 6.2 kg/m^2 and waist circumference was 88.6 cm (SD=14.1). Thirteen percent of women were current smokers. All subjects' other demographic and disease characteristics' statistics are presented in Table 5.1, for the overall population.

As shown in Table 5.2, the mean age for women at the baseline visit of CAC presence or not (CAC<10, vs. CAC≥10) was 51 (SD=2.8) and 52.2 (SD=2.4) years old, respectively (Table 5.2). The $PM_{2.5}$ annual mean levels at baseline were 16.5 (SD=1.3) and 16.6 (SD=1.3) $\mu g/m^3$, for the two CAC groups of CAC presence (<10 vs. ≥10). The other demographic and disease characteristics statistics for subgroup women with and without CAC presence (<10 vs. ≥10) at baseline visits were presented in Table 5.2. Among women with presence of CAC (N=77), 48.1% were in premenopausal or early perimenopausal status; and 41.6% were in late-perimenopausal or natural postmenopause (Table 5.2).

The unadjusted associations between each covariate and CAC presence (<10 vs. ≥10) were presented (Table 5.3). It demonstrated that the below listed variables are significant predictors of CAC, including age, BMI, morbidity (history of hypertension and diabetes), SBP, DBP, HDL-C, Ln (Triglycerides), Ln (CRP), Ln (t-PA), Ln (PAI-1), Ln (E2), Ln (HOMA). For cross-sectional analyses of baseline CAC presence (<10 vs. ≥10), the multivariable logistic regression model shows that there was no association between $PM_{2.5}$ and baseline CAC presence (Table 5.4). Further adjustment for BMI, SBP, HDL-C, Ln (CRP), Ln (PAI-1), Ln (t-PA), total physical activity scores (imputed), Ln (Trig), ln (E2), Ln (HOMA), and day of cycle were conducted in order to explore

the null findings, but that did not affect the association. More specially, with adjustment for the prior selected CVD risk factors, the relationship between $PM_{2.5}$ and CAC presence was attenuated (OR went from 1.04 to 0.99); and further adjustment for menopausal status and hormone therapy use resulted in similar effect size (OR=1.04 for the unadjusted model vs. 1.02). Although all findings remain to be non-statistically significant and the magnitude of the associations between $PM_{2.5}$ and CAC presence were similar. Both menopausal status (p for interaction=0.488) and categorical exposure time to FMP (prior/within/after 2 years of FMP) (p for interaction=0.910) did not modify the association between $PM_{2.5}$ and CAC presence (Table 5.5). The odds of CAC presence per 1 unit increase in $PM_{2.5}$ were 0.93 (95% CI: 0.64 – 1.35) in the late perimenopausal and natural post-menopausal women compared with women in premenopausal and early perimenopausal status (OR=1.34 (95% CI: 0.85 – 2.12) (Table 5.5). Interestingly, the effect sizes of CAC presence affected by $PM_{2.5}$ were larger among women in premenopausal and early perimenopausal status, compared with women in the late perimenopausal and natural post-menopauses (Table 5.5), however the 95% CI significantly overlapped. Women who had exposure measured within 2 years from FMP showed the highest ORs of CAC presence affected by $PM_{2.5}$ (OR=1.02, 95% CI: 0.69 – 1.51), compared to those who had measures taken before or after 2 years from FMP (OR=0.2, 95% CI: 0.02 – 1.88; and OR=0.37, 95% CI: 0.13 – 1.11) (Table 5.5).

For presence of CAC progression, the univariate analyses for the unadjusted associations between each covariate and presence of CAC progression (Yes vs. No) were presented (Table 5.6). In the unadjusted and adjusted multivariable logistic regression model for presence of CAC progression, the findings were similar (ORs were ranged between 1.08 and 1.12 with 95% CI included 1) and we concluded that there was no association between $PM_{2.5}$ exposure and CAC progression in this population (Table 5.7). Although the associations remain not statistically

significant, with adjustment for the prior selected covariates, the relationship between $PM_{2.5}$ and presence of CAC progression was slightly enlarged (OR went from 1.08 to 1.12) (Table 5.7). With adjustment for menopausal status and hormone therapy use, the magnitude of the association between $PM_{2.5}$ exposure and presence of CAC progression remain similar (OR=1.10 vs. 1.08 in unadjusted model) (Table 5.7). Overall, the effect sizes and significance remain similar in all those tested models for $PM_{2.5}$ and presence of CAC progression association. Both menopausal status and categorical exposure time to FMP (prior/within/after 2 years of FMP) did not modify the association between $PM_{2.5}$ and CAC progression (Table 5.8). However, the effect sizes of presence of CAC progression affected by $PM_{2.5}$ were slightly larger among late perimenopausal or natural post-menopausal status women, compared with women in premenopausal status (Table 5.8). Additionally, women who had exposure measured after 2 years from FMP showed the highest ORs of CAC progression affected by $PM_{2.5}$ (OR=1.03, 95% CI: 0.60 – 1.92), compared to those who had measures taken before or within 2 years from FMP (OR=0.62, 95% CI: 0.31 – 1.22; and OR=0.96, 95% CI: 0.62 – 1.48) (Table 5.8).

When evaluating the association between $PM_{2.5}$ and the repeated measures of CAC longitudinally, the effect sizes remain close to one and not statistically significant for all the adjusted models (Table 5.9). Additional adjustment for repeated measures of BMI, SBP, HDL, Ln (CRP), Ln (t-PA), Ln (Trig), Ln (HOMA), and time invariant physical activity score attenuated the OR (OR=1.05 for the unadjusted model vs. 0.91) (Table 5.9). Further adjustment for repeated measures of menopausal status and time invariant ever hormone therapy use attenuated the effect size even more with an OR of 0.88. Exposure time to FMP did not modify the association between $PM_{2.5}$ and repeated measures of CAC (p for interaction=0.670) (Table 5.10). The odds of repeated measures of CAC presence per 1 unit increase in $PM_{2.5}$ were 1.13 (95% CI: 0.74 – 1.75) in women

with exposure within two years of FMP; while odds of repeated measures of CAC were much smaller in women with exposure before (OR=0.61, 95% CI: 0.37 – 1.00) or after (OR= 0.51, 95% CI: 0.22 – 1.19) 2 years of FMP (Table 5.9).

We conducted several exploratory analyses in order to identify potential high-risk populations by stratifying participants' characteristics related to CVD risk. All those exploratory analyses were conducted using the final selected models (Model 5 for each outcomes), and the analyses were stratified by BMI, CRP levels, race, smoking status, age, total cholesterol, LDL, HDL. Those stratified analyses for assessing the association between $PM_{2.5}$ and CAC presence/progression detected no statistically significant findings (Appendix materials). However, the effect sizes of CAC presence and progression by $PM_{2.5}$ were larger among women who were with obesity, higher CRP, higher total cholesterol levels (≥200 mg/dL), lower HDL (<40 mg/dL), and were smokers (Appendix materials). Additionally, sensitivity analyses were conducted with rerunning the final model among women without taking any CVD medication, without any diseases, and without HRT and we detected no statistically significant findings. From those sensitivity analyses, we detected non-significant findings. However, the most evident risk of CAC presence (highest ORs) affected by $PM_{2.5}$ among women without any diseases (hypertension and diabetes mellitus); and the most evident risk of CAC progression (highest ORs) affected by $PM_{2.5}$ among women without medications taking history (cholesterol, blood pressure, or diabetes mellitus) (both had ORs=1.23) (Appendix materials). Rerunning the final models among women with imputed and observed FMP for those interaction tests related to the exposure time to FMP resulted in similar findings as among women with observed FMP (Appendix materials) (all tested p for interaction>0.4).

5.5 Discussion

Our objective was to evaluate the association between $PM_{2.5}$ ambient air pollution and CAC in middle-aged women transitioning through menopause using the SWAN Heart cohort. Overall, we found that there is no association of $PM_{2.5}$ exposure with CAC presence, detectable CAC, presence of CAC progression or repeated measures of CAC presence. Additionally, neither menopausal status nor exposure time to FMP modified the association between PM2.5 and any of CAC measures among SWAN Heart participants

Prior studies of association of the $PM_{2.5}$ exposure with CAC have yielded inconsistent findings, with most of the studies reported no overall associations as our study found (8–14). Among the seven observational studies assessed the association between ambient air pollution ($PM_{2.5}$) and CAC, the mean level of $PM_{2.5}$ exposure ranged from 13.7 to 22.8 $\mu g/m^3$ (8–14). $PM_{2.5}$ exposures of our study were in similar range (mean = 16.5, SD=1.3 $\mu g/m^3$) at lower end as those previous studies, which most of them also reported no association (8–14). The U.S. MESA and the Chinese CREATION studies reported a positive association between $PM_{2.5}$ exposure and CAC presence and progression (10,14). The MESA study assessed association of presence and progression of CAC and with $PM_{2.5}$ exposure and reported a statistically significant positive association over time (Agatston unit yearly change per IQR $PM_{2.5}$ exposure (5 $\mu m/m^3$) increase: 4.1 (95% CI: 1.4 to 6.8)) (10). Their large sample size (N=6834 had ≥2 CAC) with longer period of follow-up for repeated measures of CAC (mean follow-up time for the tow CAC = 6.2 years) might provide sufficient time allowing CAC to progress over time. In addition, such large sample size also provides large power to capture the positive association if there is any. Our sample size (N=~350) might not provide enough power to detect the association. In addition, the Chinese cross-sectional study, by Wang et al. estimated the long-term exposure to ambient $PM_{2.5}$ and reported a

positive association between long-term $PM_{2.5}$ exposure and CAC (Agatston unit % increase per $PM_{2.5}$ exposure IQR increase (30 μm/m^3): 27.2 % (95% CI: 10.8 to 46.1%)) (14). They concluded that $PM_{2.5}$ exposure was independently positively associated with CAC (14). Even though both of our and this Chinese study used same study design, we included different race/ethnicity group (white and black), and as our exposure levels were much lower than the Chinese study (16.5±1.3 μg/m^3 for our study vs. 70.1±20.0 μg/m^3 for the Chinese study). These may result in our different (null) findings than theirs (positive associations). Our results add to prior findings evaluating associations of $PM_{2.5}$ exposure and CAC by evaluating the association among the middle-aged women transiting through menopause. Our analyses did not find evidence of associations of the $PM_{2.5}$ exposure with CAC presence and progression, which are in agreement with most of the previous studies in other population (e.g. MESA, German HNR).

Our investigation is the first one focused on the effect modification of menopausal status on the association between long-term $PM_{2.5}$ exposure with CAC presence and progression. As mentioned above, except our study, there is only one study conducted so far on association between long-term $PM_{2.5}$ exposure and CAC presence that further evaluated the association among postmenopausal women. The study by Wang et al, 2019 reported that a stronger effect of $PM_{2.5}$ to CAC were found in sensitivity analyses including postmenopausal women (Percent change in CAC associated with $PM_{2.5}$ (per 30 μg/m^3): 34.5%, 95% CI: 5.8 to 70.9%), compared to the general population (with smaller effect size of 27.2 %, 95% CI: 10.8 to 46.1%) (14). We cannot tell whether this stronger association might be due to aging or effect of menopausal transition in this Chinese study, as their study only included postmenopausal women for the subgroup analyses. In order to disentangle the effect of chronological and menopausal transition on this association of $PM_{2.5}$ and CAC, data of menopausal status and factors changes during menopausal transition over

time are required. In our study utilizing data from the SWAN heart study, we found the stronger association between long-term $PM_{2.5}$ exposure and presence of CAC among women at premenopausal and early peri-menopausal status (OR=1.34, 95% CI: 0.85 – 2.12), than women who were at late peri-menopausal and natural postmenopausal status (OR= 0.93, 95% CI: 0.64 – 1.35) (both were non-significant findings) (Table 5.5), this could be influenced by the fact that women at baseline were more likely to be at an earlier stage of the menopausal transition.

Additionally, when we evaluating the exposure measure time to FMP, the magnitude of association remained similar close to one and significance remained not significant. Although, we detected the strongest effect of long-term $PM_{2.5}$ exposure to CAC presence showing among women who had their exposure measured within 2 years of FMP (OR=1.02, 95% CI: 0.69 – 1.51), compared with those who had exposure measured 2 years before (OR=0.20, 95% CI: 0.02 – 1.88) and after (OR=0.37, 95% CI: 0.13 – 1.11) FMP. The time around FMP is a critical period when dynamic metabolic and physiological changes occur. Our findings of the strongest effect of long-term $PM_{2.5}$ exposure to CAC presence shown among those women who had exposure closest to the menopause (within 2 years of FMP) indicate a possible interplay between changes during the menopausal transition (e.g. hormone dysregulation) and air pollution on CAC presence. This is supported by previous studies showing that various changes were detected around FMP (e.g. some women experienced patterns of estradiol decline, or follicle-stimulating hormones rise over the menopause transition, but not all women) (93). Although, when we further adjusted for hormone level (e.g. E2) for assessing the association between $PM_{2.5}$ exposure to CAC presence, the effect size and significance remained similar as the model without adjusting E2. Further research with larger sample size in each of the time to FMP subgroup is needed to confirm this finding. Additionally, when we looked at the association between $PM_{2.5}$ exposure to presence of CAC

progression, we reported that women who had exposure measured after 2 years from FMP showed the highest OR of CAC progression affected by $PM_{2.5}$ (OR=1.03, 95% CI: 0.60 – 1.92), compared to those who had measures taken before or within 2 years from FMP (OR=0.62, 95% CI: 0.31 – 1.22; and OR=0.96, 95% CI: 0.62 – 1.48). As the presence of CAC progression was defined by our study using both baseline and follow-up CAC scores, and those women who had exposure measured after 2 years from FMP would have their endpoint outcome, CAC progression, even later than after 2 years of FMP. A possible reason for the higher detected ORs of presence of CAC progression could potentially due to the much older age of those women, which partially supports the age effect. This finding, although not significant and we looked at the CAC progression here, is somewhat consistent with what the above-mentioned Chinese study, by Wang et al., 2019 reported (14), which looked at only the CAC presence due to its cross-sectional design. It is worthwhile to note that we should keep in mind not to over interpret these non-significant findings; especially there is actually some overlap in the reported 95% CIs.

To the best of our knowledge, no study has evaluated the effect of menopausal transition on the association between $PM_{2.5}$ and CAC progression. In our study, we found no association between $PM_{2.5}$ and CAC progression, with detecting a non-significant slightly stronger association between long-term $PM_{2.5}$ exposure and presence of CAC progression among women at late peri-menopausal and natural post-menopause status (OR=1.08, 95% CI: 0.74 – 1.58), compared to women who were at premenopausal and early peri-menopausal status (OR=1.01, 95% CI: 0.65 – 1.57), however the 95% CI significantly overlapped indicating this difference to not be meaningful.

Future research should explore further whether some of those factors (e.g. CRP, SBP) in women transitioning through menopause are the potential pathways linking the association

between $PM_{2.5}$ and CAC, using a larger sample size of population in a longitudinal study design. Although our findings supported that menopausal transition were not effect modifiers on the association between $PM_{2.5}$ and repeated measures of CAC. A non-significant stronger effect size of repeated measures of CAC effected by long-term $PM_{2.5}$ was detected among women who had exposure measurement within 2 years of FMP (OR=1.13, 95% CI: 0.74 – 1.75), compared with those who had exposure measured 2 years before (OR=0.61, 95% CI: 0.37 – 1.00) and after (OR=0.51, 95% CI: 0.22 – 1.19) FMP (Table 5.10). Those consistently detected higher risks of CAC progression effected by $PM_{2.5}$ among peri-menopausal or natural postmenopause women (or time close to FMP) highlighted the potential critical time around menopause as a period when critical changes in vasculature and CVD risk factors accumlate, in which women during this period might be affected by $PM_{2.5}$ more than other time. Overall, although those above-mentioned findings of the subgroup analyses were not statistically significant, the detected stronger effect sizes are important to draw our attention, as they could be more sensitive group to target on for CVD prevention. Future work needs to be done using larger sample size of women during menopausal transition to allow enough power to detect the potential associations.

Our study did not detect association between $PM_{2.5}$ and CAC overall, however, our subgroup analyses and sensitivity analyses showed that the effect size of CAC presence and progression affected by long-term $PM_{2.5}$ were larger among subgroups of women who were obese, or have higher CRP, higher total cholesterol levels, and lower HDL. These results support the idea that the possible pathways of the chronic effect of long-term PM_2 on SCA, measured by CAC, could be through the effect on systemic oxidative stress that increases lipids (higher effect size detected among higher lipid levels' group) and systematic inflammation (higher effect size detected among higher CRP group). The effect of $PM_{2.5}$ to atherosclerosis could be through

reactive oxygen species (ROS), which regulates the signaling pathways and vascular biology (2). In addition, stimulate of $PM_{2.5}$ may lead to endothelial dysfunction, with detrimental effect on vascular cells (2–5), which could potentially accelerate the progression of atherosclerosis. Adjusting for CVD risk factors and potential mediators is not the optimal method for model building, but we adjusted those risk factors, as those are modified during menopausal transition and we wanted to explore how those factors affect the association between long-term $PM_{2.5}$ and CAC presence and progression.

Although our sensitivity analyses detected non-significant findings, the most evident risk of CAC affected by $PM_{2.5}$ were detected among women without any diseases (hypertension and diabetes mellitus); and those without medications taking history (cholesterol, blood pressure, or diabetes mellitus). Our findings adds to the lines of evidence that among those taking medications (e.g. blood pressure medication), the effect of $PM_{2.5}$ on adverse cardiovascular outcome appeared to be mitigated (215), which partially explaining the age effect, as younger women were less likely to take those medications. Additionally, given that midlife women who had pre-existing disease (e.g. hypertension and diabetes mellitus) or took medications (e.g. medications for lowering cholesterol or blood pressure) are those who have to take actions for taking care of their cardiovascular health, it is possible that those actions' effects help with reducing the impact of $PM_{2.5}$ to CAC among those.

Further study is needed to confirm this by looking into how those medications, for example, might dilute the effect of $PM_{2.5}$ on CAC. Our finding provides evidence that woman who had not prior health issues might be affected by air pollution even more on their CAC. These results imply the importance of raising all middle-aged women's awareness of the adverse cardio-metabolic

health changes during their midlife due to adverse environmental exposure, especially for those women without any pre-existing disease or medication taking history.

Strengths and limitations

The study has several limitations. Because the study design and analyses are observational, there is potential for residual confounding. However, we have adjusted for many combinations of potential confounders during our modelling building process, and the effect sizes were all remain close to one and not statistically significant overall. In addition, as we do not have detailed long-term follow-up information on participants' residential history, we were unable to study the day-to-day activities' related exposure and the long-term exposure window, which could also have effects to CAC. Since atherosclerosis is a chronic disease, and takes time to progress eventually, exposure over a long period might have a greater impact on the disease progress than the recent exposures. Therefore, the repeated measures of outcomes conducted within an average of 2 years in our cohort may not allow enough time for progression of CAC to occur, which might be the reason why we did not capture any association between $PM_{2.5}$ and CAC progression. However, longer time than 2-3 years would not allow us to observe changes occurring during menopausal transition to study how those may affect the progression of CAC. Furthermore, considering that our study sample size was relatively small (~ 350 women), we need to interpret the findings with cautions, especially for those null findings and those ORs with very wide confidence intervals. This limited our ability to assess study aims (e.g. interaction) in each categorical group, especially those ones with even smaller sample size (e.g. peri-menopausal women). Replication of this work in other larger groups of women would be needed to confirm our findings. Last, the study-included women are mostly white and well educated; the generalizability of these data to other more diverse populations is limited. Although, this SWAN heart study cohort collected many data capturing

changes of middle-age women as they approach the menopause, allowing us to evaluate associations extensively with testing interesting interactions.

The study has several strengths. We used many ways to categorize CAC based on the clinical relevance and what other studies have been used, and they are CAC presence, detectable CAC, presence of CAC progression, and repeated measures of CAC presence. As CAC Agatston score in observational study has right-skewed distribution due to many zero scores, and statistically it is challenge to treat CAC score as simply a continuous variable or as a categorical variable when building statistical models. Those various ways (two ways for CAC presence; one way for longitudinal repeated measures of CAC, and one way for CAC progression) we used for categorizing CAC presence and progression have been tested and validated in the previous studies, but not on the same topic. Our thoroughly evaluations used four different ways to treat out outcome of interest provide us confidence on our reported findings. Another strength of our study is that the SWAN heart study included well-recorded clinical review, biochemical and radiological data allowing us to evaluate those women' various physiological profiles at their mid-life. As the atherosclerosis is the early stage of cardiovascular disease, being able to notice the potential cardiovascular health issues of women during their midlife, which is affected by the air pollution, is key to reducing cardiovascular risk among women later in life. Our effort of identifying women who could be at higher risk of atherosclerosis due to air pollution will help them take actions to reduce their risks of CAC by paying attention to their polluted air exposure, as they are the vulnerable population.

5.6 Conclusion

In conclusion, we observed no evidence that $PM_{2.5}$ exposure is associated with the CAC presence or progression among middle-aged women. However, stronger effect of $PM_{2.5}$ to presence of CAC progression was detected among subgroups of women, such as those ones who were in late peri-menopausal and natural postmenopausal status or without taking CVD medications. Those women could be more sensitive group impacted by $PM_{2.5}$ to CAC and raising their awareness of the adverse cardio-metabolic health changes during their midlife due to adverse environmental exposure is important. These findings add to existing evidence on the association of the $PM_{2.5}$ exposure and CAC, with a focusing on the middle-aged women.

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5.8 Tables and Figures

Table 5.1 Characteristics of study participants at baseline (N=366)

Characteristics	Total N, %
All	366, 100%
White	238, 65%
Smoker	48, 13.1%
Education Level	
Less than college degree	57, 15.6%
Some College/College Degree	189, 51.6%
Post College	120, 32.8%
Family Income (75,000+: Yes vs. No)	135, 36.9%
Ever used hormone therapy (Yes vs. No)	107, 29.2%
Menopause status ^^	
Pre- and Early peri-menopausal	198, 54.1%
Late peri-menopausal and Natural post	129, 35.2%
Medication use (Yes vs. No) ‡	83, 22.7%
Morbidity (Yes vs. No) *	111, 30.3%
Categorical "Time to FMP" (cutoff: 1 year) ‡‡	
Pre: $PM_{2.5}$ exposure date to FMP date time interval <-1	131, 35.8%
Mid: $-1 \leq PM_{2.5}$ exposure date to FMP date time interval ≤ 1	63, 17.2%
Post: $PM_{2.5}$ exposure date to FMP date time interval>1	62, 16.9%
Categorical "Time to FMP" cutoff: 2 years) **	
Pre: $PM_{2.5}$ exposure date to FMP date time interval < -2	101, 27.6%
Mid: $-2 \leq PM_{2.5}$ exposure date to FMP date time interval ≤ 2	112, 30.6%
Post: $PM_{2.5}$ exposure date to FMP date time interval > 2	43, 11.7%

Table 5.1 Continued

CAC presence	
CAC<10	289, 79%
CAC≥10	77, 21%
Detectable CAC	
CAC=0	194, 53%
CAC>0	172, 47%
Presence of CAC progression^	
No	200, 79.1%
Yes	53, 20.9%
	Mean (SD)
CAC score visit 1 **median (Q1-Q3)**	0 (0-6.2)
CAC score visit 2 **medians (Q1-Q3)**	0 (0-10.3)
$PM_{2.5}$ annual mean level (µg/m3)	16.5 (1.3)
Time since FMP **median (Q1-Q3)** ‡‡	-1.1 (3.3-0.8)
Age at SWAN heart baseline (years)	51.3 (2.8)
BMI (kg/m^2)	29 (6.2)
Waist (cm)	88.6 (14.1)
Physical activity total score†	7.9 (1.7)
Systolic blood pressure (SBP) (mmHg)	117.8 (16.9)
Diastolic blood pressure (DBP) (mmHg)	75 (10)
Cholesterol (mg/dL)	201.4 (37.9)
High-density lipoprotein cholesterol (HDL-C) (mg/dL)	57.5 (14.4)
Low-density lipoprotein cholesterol (LDL-C) (mg/dL)	119.8 (33)
Triglyceride (mg/dL), **median (Q1-Q3)**	99 (76-138)
Follicle-stimulating hormone (FSH) (mIU/mL), **median (Q1-Q3)**	36 (15.4-79.8)
Estradiol (E2) (pg/mL), **median (Q1-Q3)**	33.9 (16.2-79.6)
C-reactive protein (CRP) (ng/dL), **median (Q1-Q3)**	2.4 (0.9-7.2)
HOMA, **median (Q1-Q3)**	1.9 (1.4-3.1)

Table 5.1 Continued

Plasminogen activator inhibitor-1 (PAI-1) (ng/dL), **median (Q1-Q3)**	13.3 (7.6-24.4)
Tissue plasminogen activator Antigen (t-PA) (ng/dL), **median (Q1-Q3)**	6.8 (5.2-9.3)

BMI, body mass index; CAC: coronary artery calcification; FMP, final menstrual period; FSH, follicle-stimulating hormone; and HOMA, homeostasis model assessment.
^^Menopausal status had 39 women (10.7% of the 366 women) with missing data, as those women had surgical menopause, therefore, were coded into missing and excluded from the analyses related to menopause status
‡Taking medications for cholesterol, blood pressure, or diabetes mellitus
**Morbidity: history of hypertension and diabetes mellitus*
^Presence of CAC progression had 253 women total, as those are the ones with two time points of CAC measures
‡‡Time to FMP (cutoff: 1 year) had 110 women (30.1%) with missing data, as those women had no observed FMP
*** Time to FMP (cutoff: 2 years) had 110 women (30.1%) with missing data, as those women had no observed FMP*
†Modified Baecke Scores of habitual physical activity: higher scores indicating more physical activity

Table 5.2 Characteristics of study participants at baseline by CAC presence (<10 vs. ≥10) (N=366)

Characteristics	CAC<10, 2001-2004 N, %	CAC≥10, 2001-2004 N, %	Chi-squared or Fisher' exact test p-value
All	289, 79%	77, 21%	
White	189, 65.4%	49, 63.6%	0.773
Smoker	36, 12.5%	12, 15.6%	0.468
Education Level			0.294
Less than college degree	41, 14.2%	16, 20.8%	
Some College/College Degree	154, 53.3%	35, 45.5%	
Post College	94, 32.5%	26, 33.8%	
Family Income (75,000+: Yes vs. No)	111, 38.4%	24, 31.2%	0.254
Ever used hormone therapy (Yes vs. No)	84, 29.1%	23, 29.9%	0.890
Menopause status^^			0.185
Pre- and Early peri-menopausal	161, 55.7%	37, 48.1%	
Late peri-menopausal and Natural post	97, 33.6%	32, 41.6%	
Medication use (Yes vs. No) ‡	62, 21.5%	21, 27.3%	0.279
Morbidity (Yes vs. No)	77, 26.6%	34, 44.2%	0.003
Categorical "Time to FMP" (cutoff: 1 year) ‡‡			0.083
Pre: $PM_{2.5}$ exposure date to FMP date time interval <-1	109, 37.7%	22, 28.6%	
Mid: $-1 \leq PM_{2.5}$ exposure date to FMP date time interval ≤ 1	44, 15.2%	19, 24.7%	
Post: $PM_{2.5}$ exposure date to FMP date time interval>1	46, 15.9%	16, 20.8%	
Categorical "Time to FMP" cutoff: 2 years) **			
Pre: $PM_{2.5}$ exposure date to FMP date time interval < -2	84, 29.1%	17, 22.1%	0.172
Mid: $-2 \leq PM_{2.5}$ exposure date to FMP date time interval ≤ 2	85, 29.4%	27, 35.1%	
Post: $PM_{2.5}$ exposure date to FMP date time interval > 2	30, 10.4%	13, 16.9%	

Table 5.2 Continued	**Mean (SD)**	**Mean (sd)**	**p-value for T test or Wilcoxon-Mann-Whitney test***
$PM_{2.5}$ annual mean level ($\mu g/m^3$)	16.5 (1.3)	16.6 (1.4)	0.656
Age at SWAN heart baseline (years)	51 (2.8)	52.2 (2.4)	0.000
BMI (kg/m^2)	27.7 (5.1)	34.3 (7.5)	<.0001
Waist (cm)	85.5 (11.7)	100.5 (16.3)	<.0001
Physical activity total score‡	8 (1.8)	7.4 (1.6)	0.017
Systolic blood pressure (SBP) (mmHg)	115.9 (15.7)	125.1 (19.1)	0.000
Diastolic blood pressure (DBP) (mmHg)	74.3 (9.9)	77.6 (10.2)	0.013
Cholesterol (mg/dL)	199.8 (37.4)	207.4 (39.1)	0.117
High-density lipoprotein cholesterol (HDL-C) (mg/dL)	58.6 (14.3)	53.3 (13.7)	0.003
Low-density lipoprotein cholesterol (LDL-C) (mg/dL)	118.8 (32.9)	123.5 (33.1)	0.271
Triglyceride (mg/dL), **median (Q1-Q3)**	99 (73-131)	106 (84-189)	0.008
Follicle-stimulating hormone (FSH) (mIU/mL), **median (Q1-Q3)**	35.4 (15.1-83.7)	39.7 (18.3-73.6)	0.293
Estradiol (E2) (pg/mL), **median (Q1-Q3)**	40 (16.2-98.1)	25.2 (16.2-44.2)	0.018
C-reactive protein (CRP) (ng/dL), **median (Q1-Q3)**	2 (0.8-5.4)	5.3 (1.4-12.3)	<.0001
HOMA, **median (Q1-Q3)**	1.8 (1.4-2.7)	3 (1.8-4.9)	<.0001
Plasminogen activator inhibitor-1 (PAI-1) (ng/dL), **median (Q1-Q3)**	11.9 (7.3-22.7)	19.8 (9.8-33.2)	0.142
Tissue plasminogen activator Antigen (t-PA) (ng/dL), **median (Q1-Q3)**	6.7 (5-8.8)	7.8 (6-10.6)	0.264

BMI, body mass index; CAC: coronary artery calcification; FMP, final menstrual period; FSH, follicle-stimulating hormone; and HOMA, homeostasis model assessment.

Table 5.2 Continued

^^ Menopausal status had 39 women with missing data, as those women had surgical menopause, therefore, were coded into missing and excluded from the analyses related to menopause status
‡Taking medications for cholesterol, blood pressure, or diabetes mellitus
**Morbidity: history of hypertension and diabetes mellitus*
‡‡Time to FMP (cutoff: 1 year) had 110 women (30.1%) with missing data, as those women had no observed FMP
*** Time to FMP (cutoff: 2 years) had 110 women (30.1%) with missing data, as those women had no observed FMP*
†Modified Baecke Scores of habitual physical activity: higher scores indicating more physical activity

Table 5.3 Univariate regression: Associations between baseline study variables and presence of CAC (CAC<10 vs. CAC≥10) in women at Midlife (N=366)

Variables	Odds Ratio	95% Confidence Interval	p-value
Race (White vs Black)	1.08	0.64 - 1.82	0.773
Smoking (Yes vs. No)	1.30	0.64 - 2.65	0.469
Education Level (Ref: Less than college degree)			0.298
Some College/College Degree	0.58	0.29 - 1.15	
Post College	0.71	0.34 - 1.46	
Family income (>= $75,000 vs. < $75,000)	0.73	0.42 - 1.26	0.255
Medication use‡	1.37	0.77 - 2.44	0.280
Morbidity *	2.18	1.29 - 3.66	0.003
Ever used hormone therapy (Yes vs. No)	1.04	0.60 - 1.80	0.890
Menopause status (Ref: Pre- and Early peri-menopausal)			
Late peri-menopausal & Natural Post	1.44	0.84 - 2.45	0.186
Categorical "Time to FMP" (cutoff: 2 years)			0.177
Mid: $-2 \leq PM_{2.5}$ exposure date to FMP date time interval ≤ 2	1.57	0.80 - 3.09	
Post: $PM_{2.5}$ exposure date to FMP date time interval > 2	2.14	0.93 - 4.93	
Time of $PM_{2.5}$ exposure to FMP	1.08	0.99 - 1.19	0.095

Table 5.3 Continued

Age at Visit	1.17	1.07 - 1.28	0.001
Physical activity total score†	0.81	0.70 - 0.94	0.007
BMI	1.19	1.13 - 1.25	<.0001
Systolic blood pressure (SBP)	1.03	1.02 - 1.05	<.0001
Diastolic blood pressure (DBP)	1.03	1.01 - 1.06	0.012
Cholesterol	1.01	1.00 - 1.01	0.118
High-density lipoprotein cholesterol (HDL-C)	0.97	0.95 - 0.99	0.004
Low-density lipoprotein cholesterol (LDL-C)	1.00	1.00 - 1.01	0.270
Ln (Triglyceride)	2.34	1.40 - 3.91	0.001
Ln (CRP)	1.53	1.24 - 1.87	<.0001
Ln (PAI-1)	1.61	1.22 - 2.13	0.001
Ln (t-PA)	2.19	1.29 - 3.72	0.004
Ln (E2)	0.74	0.57 - 0.95	0.017
Ln (FSH) (mIU/mL)	0.99	0.76 - 1.28	0.938
Ln (HOMA)	3.04	1.95 - 4.76	<.0001

BMI, body mass index; CAC: coronary artery calcification; FMP, final menstrual period; FSH, follicle-stimulating hormone; and HOMA, homeostasis model assessment.
‡Taking medications for cholesterol, blood pressure, or diabetes mellitus
**Morbidity: history of hypertension and diabetes mellitus*
†Modified Baecke Scores of habitual physical activity: higher scores indicating more physical activity

Table 5.4 Association between $PM_{2.5}$ and presence of CAC (<10 vs. ≥10)

Per 1 µg/m3 increase in $PM_{2.5}$	N	Overall OR (95% CI)
Unadjusted model	366	1.04 (0.86 - 1.26)
Model 1	366	1.04 (0.86 - 1.26)
Model 2	366	1.06 (0.87 - 1.28)
Model 3	276	0.99 (0.77 - 1.27)
Model 4	251	1.02 (0.74 - 1.41)
Model 5	309	1.05 (0.83 - 1.32)

**Model 1: adjusted for site;*
Mode 2: model 1+age, race, study site, income;
Model 3: model 2+selected covariates (BMI, SBP, HDL, Ln (CRP), Ln (PAI-1), Ln (t-PA), total physical activity scores (imputed), Ln (Trig), ln (E2), Ln (HOMA), Day of cycle);
Model 4: model 3+menopause status, and hormone therapy use;
Model 5: adjusted for age, race, study site, imputed physical activity score, BMI, SBP, Ln (CRP), Ln (HOMA)

Table 5.5 Effect of $PM_{2.5}$ on presence of CAC (<10 vs. ≥10) in the stratified groups (by menopausal status and exposure time to FMP (cutoff 2 years))

Presence of CAC (≥10)	Pre- and Early peri-menopausal		Late peri-menopausal& Natural Post-menopausal		p for interaction
	N	OR (95% CI)	N	OR (95% CI)	
Per 1 μg/m^3 increase in $PM_{2.5}$	162	1.34 (0.85 - 2.12)	113	0.93 (0.64 - 1.35)	0.488

	Pre: > 2 y Before FMP		Mid: Within 2 y of FMP		Post: > 2 y After FMP		p for interaction
	N	OR (95% CI)	N	OR (95% CI)	N	OR (95% CI)	
Per 1 μg/m^3 increase in $PM_{2.5}$	83	0.20 (0.02 - 1.88)	92	1.02 (0.69 - 1.51)	37	0.37 (0.13 - 1.11)	0.910

** The above subgroup analyses were conducted using the final selected model, model 5($PM_{2.5}$ and presence of CAC), which adjusted for age, race, study site, imputed physical activity score, BMI, SBP, Ln (E2), Ln (CRP), Ln (HOMA);*

For subgroup of postmenopausal women, additional variable, hormones therapy ever use, also was included in the model.

Table 5.6 Univariate regression: Associations between baseline study variables and presence of CAC progression in women at Midlife (N=253)

Variables	Odds Ratio	95% Confidence Interval	p-value
Race (White vs Black)	1.12	0.59 - 2.13	0.733
Smoking (Yes vs. No)	1.30	0.52 - 3.26	0.573
Education Level (Ref: Less than college degree)			0.651
Some College/College Degree	1.24	0.52 - 2.97	
Post College	0.90	0.34 - 2.37	
Family income (>= $75,000 vs. < $75,000)	1.16	0.62 - 2.20	0.643
Medication use‡	1.83	0.94 - 3.58	0.077
Morbidity*	2.48	1.32 - 4.65	0.005
Ever used hormone therapy (Yes vs. No)	0.74	0.37 - 1.48	0.397
Menopause status (Ref: Pre- and Early peri-menopausal)			
Late peri-menopausal & Natural Post	1.26	0.66 - 2.38	0.486
Categorical "Time to FMP" (-1, -1~1, 1)			0.642
Mid: $-1 \leq PM_{2.5}$ exposure date to FMP date time interval ≤ 1	1.29	0.57 - 2.94	
Post: $PM_{2.5}$ exposure date to FMP date time interval >1	1.46	0.64 - 3.36	

Table 5.6 Continued

Categorical "Time to FMP" (-2, -2~2, 2)			0.342
Mid: -2 ≤ $PM_{2.5}$ exposure date to FMP date time interval ≤ 2	0.74	0.34 - 1.58	
Post: $PM_{2.5}$ exposure date to FMP date time interval > 2	1.47	0.58 - 3.73	
Age at Visit	1.08	0.96 - 1.21	0.207
Physical activity total score†	0.84	0.70 - 1.00	0.054
BMI	1.08	1.03 - 1.13	0.002
Systolic blood pressure (SBP)	1.02	1.00 - 1.04	0.087
Diastolic blood pressure (DBP)	1.03	0.99 - 1.06	0.137
Cholesterol	1.01	1.00 - 1.02	0.006
High-density lipoprotein cholesterol (HDL-C)	0.98	0.95 - 1.00	0.062
Low-density lipoprotein cholesterol (LDL-C)	1.01	1.00 - 1.02	0.019
Ln (Triglyceride)	2.16	1.18 - 3.96	0.013
Ln (CRP)	1.10	0.87 - 1.39	0.427
Ln (PAI-1)	2.15	1.52 - 3.05	<.0001
Ln (t-PA)	2.18	1.06 - 4.51	0.035
Ln (E2)	0.89	0.67 - 1.18	0.434
Ln (FSH) (mIU/mL)	0.90	0.66 - 1.22	0.493
Ln (HOMA)	1.69	0.97 - 2.95	0.066

Table 5.6 Continued

BMI, body mass index; CAC: coronary artery calcification; FMP, final menstrual period; FSH, follicle-stimulating hormone; and HOMA, homeostasis model assessment.
Taking medications for cholesterol, blood pressure, or diabetes mellitus
Morbidity: history of hypertension and diabetes mellitus
Modified Baecke Scores of habitual physical activity: higher scores indicating more physical activity

Table 5.7 Association between $PM_{2.5}$ and presence of CAC progression (Yes, vs. No)

Per 1 $\mu g/m^3$ increase in $PM_{2.5}$	Overall	
	N	OR (95% CI)
Unadjusted model	253	1.08 (0.84 - 1.39)
Model 1	253	1.09 (0.85 - 1.40)
Model 2	231	1.11 (0.86 - 1.43)
Model 3	208	1.12 (0.85 - 1.47)
Model 4	189	1.10 (0.80 - 1.51)
Model 5	245	1.07 (0.83 - 1.38)

**Model 1: adjusted for site;*
Model 2: model 1+age, race, study site, income;
Model 3: model 2+selected covariates (BMI, LDL, Ln (PAI-1), Ln (t-PA), Ln (Triglyceride);
Model 4: model 3+menopause status, and hormone therapy use;
Model 5: adjusted for age, study site, race, BMI, and total cholesterol (Based on the univariate analysis, table 5.6, the levels of total cholesterol, Ln (Triglyceride) and LDL were all statistically significant different between presence of CAC progression Yes and. No group, however we cannot include all those variables into the same model due to the collinearity issues as described in the method section. Therefore, for model 3, we included LDL and Ln (Triglyceride) in the model to assess whether those change the effect size. Later on, in the stepwise model selection, when we added all three variables into the model, as they all were statistically significant, the total cholesterol was the one kept in the model. The removal of other two variables did not change the effect estimates much).

Table 5.8 Effect of $PM_{2.5}$ on presence of CAC progression in the stratified groups (by menopausal status and exposure time to FMP (cutoff 2 years))

Presence of CAC progression (Yes)	Pre- and Early peri-menopausal		Late peri-menopausal & Natural Post-menopausal		p for interaction
	N	OR (95% CI)	N	OR (95% CI)	
Per 1 µg/m^3 increase in $PM_{2.5}$	133	1.01 (0.65 - 1.57)	84	1.08 (0.74 - 1.58)	0.581

	Pre: > 2 y Before FMP		Mid: Within 2 y of FMP		Post: > 2 y After FMP		p for interaction
	N	OR (95% CI)	N	OR (95% CI)	N	OR (95% CI)	
Per 1 µg/m^3 increase in $PM_{2.5}$	66	0.62 (0.31 - 1.22)	78	0.96 (0.62 - 1.48)	29	1.03 (0.60 - 1.92)	0.422

** The above subgroup analyses were conducted using the final selected model, model 5($PM_{2.5}$ and presence of CAC progression), which adjusted for age, study site, race, BMI, total cholesterol;*

For subgroup of postmenopausal women, additional variable, hormones therapy ever use, also was included in the model.

Table 5.9 Association between $PM_{2.5}$ and CAC repeated measures

Per 1 μg/m^3 increase in $PM_{2.5}$	N	Overall (Repeated measures of CAC) OR (95% CI)
Unadjusted model	619	1.05 (0.89 - 1.24)
Model 1	619	1.04 (0.88 - 1.23)
Model 2	562	1.08 (0.90 - 1.28)
Model 3	459	0.91 (0.74 - 1.12)
Model 4	413	0.88 (0.69 - 1.12)
Model 5	573	0.94 (0.78 - 1.14)

**Model 1: adjusted for site;*
Mode 2: model 1+age, race, study site, income;
Model 3: model 2+selected covariates (BMI, SBP, HDL, Ln (CRP), Ln (t-PA), Ln (Trig), Ln (HOMA), physical activity score (imputed);
Model 4: model 3+menopause status, and hormone therapy use;
Model 5: adjusted for age, study site, race, and time varying: BMI, HDL, and SBP.

Table 5.10 Effect of $PM_{2.5}$ on CAC repeated measures in the stratified groups (by menopausal status and exposure time to FMP (cutoff 2 years))

Repeated CAC measures	Pre: > 2 y Before FMP		Mid: Within 2 y of FMP		Post: > 2 y After FMP		p for interaction
	N	OR (95% CI)	N	OR (95% CI)	N	OR (95% CI)	
Per 1 $\mu g/m^3$ increase in $PM_{2.5}$	157	0.61 (0.37 - 1.00)	180	1.13 (0.74 - 1.75)	68	0.51 (0.22 - 1.19)	0.203

** The above subgroup analyses were conducted using the final selected model, model 5($PM_{2.5}$ and CAC repeated measures), which adjusted for age, study site, race, and time varying: BMI, HDL, and SBP.*

For subgroup of postmenopausal women, additional variable, hormones therapy ever use, also was included in the model.

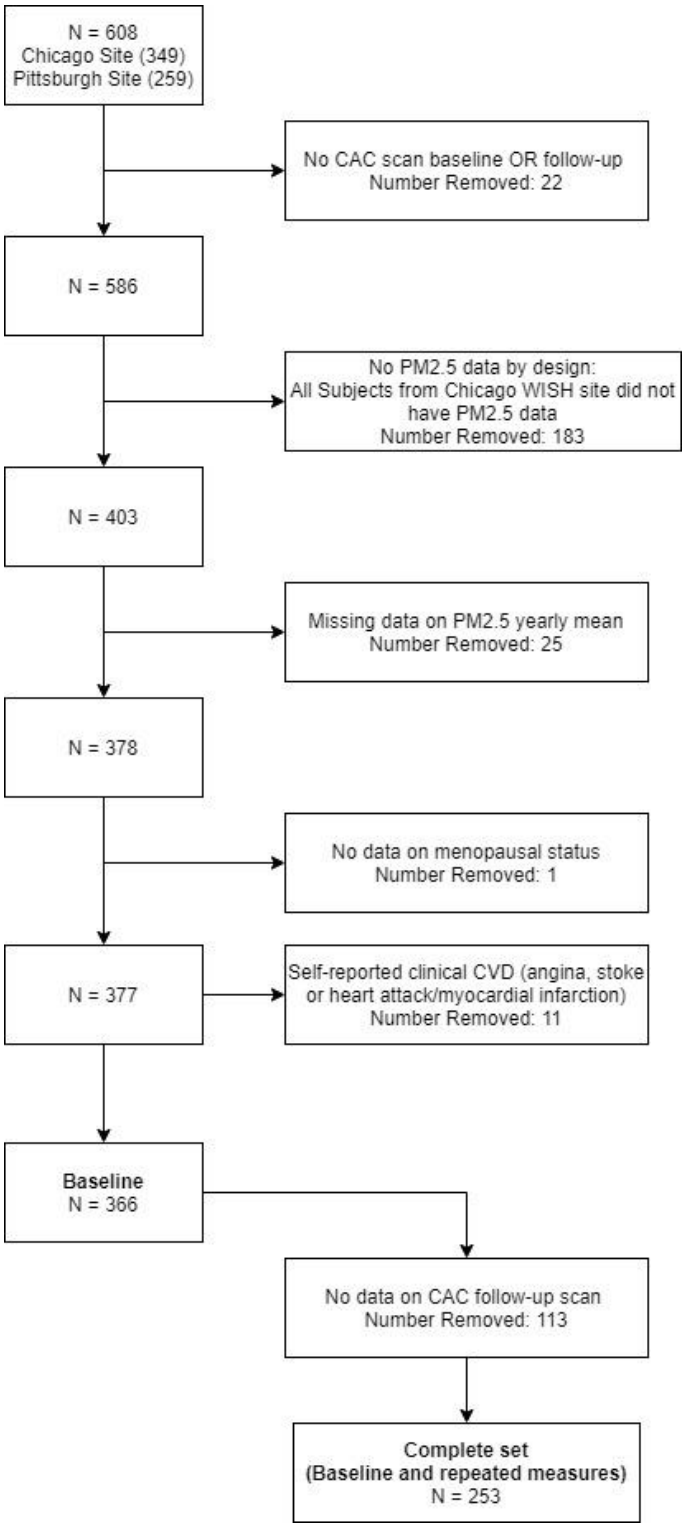


Figure 5.1 Participant flow chart, main association between $PM_{2.5}$ and CAC presence/progression, SWAN Heart study, 2001-2006

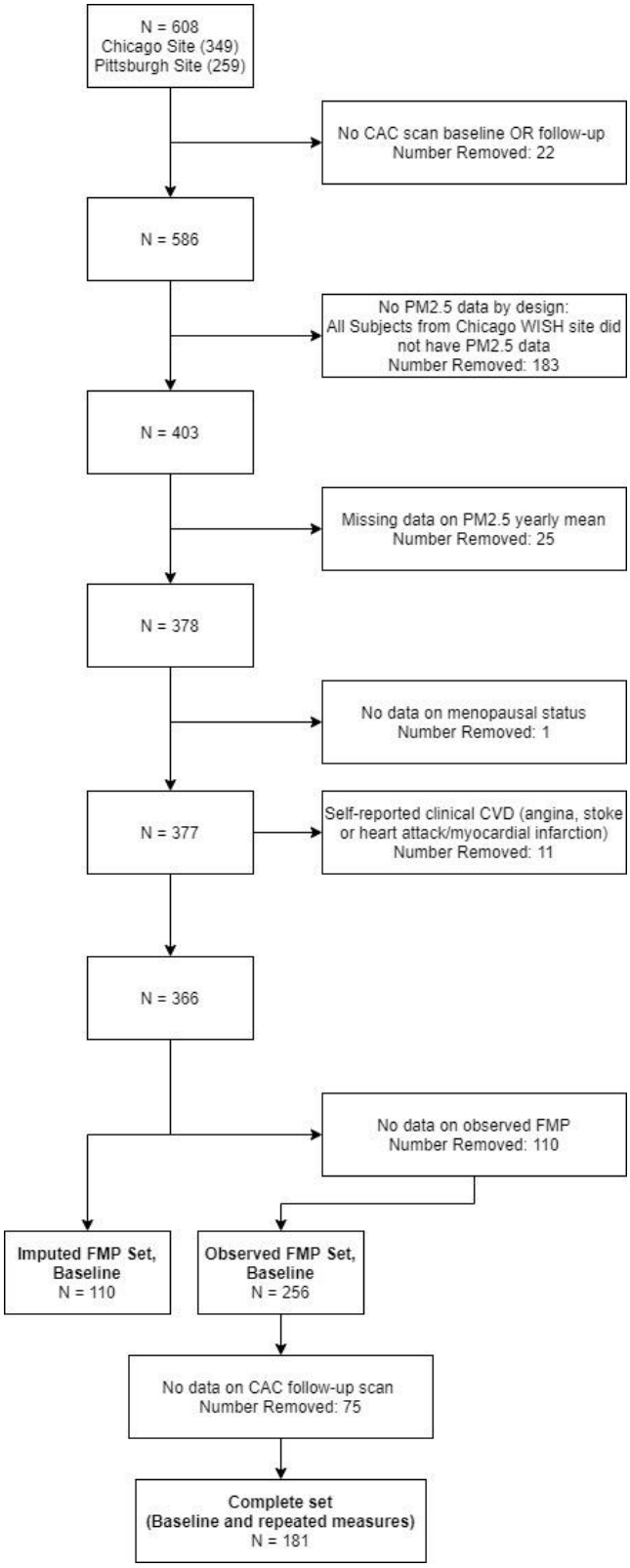


Figure 5.2 Participant flow chart: effect modification of time since FMP on the association between $PM_{2.5}$ and CAC presence/progression, SWAN Heart study, 2001-2006

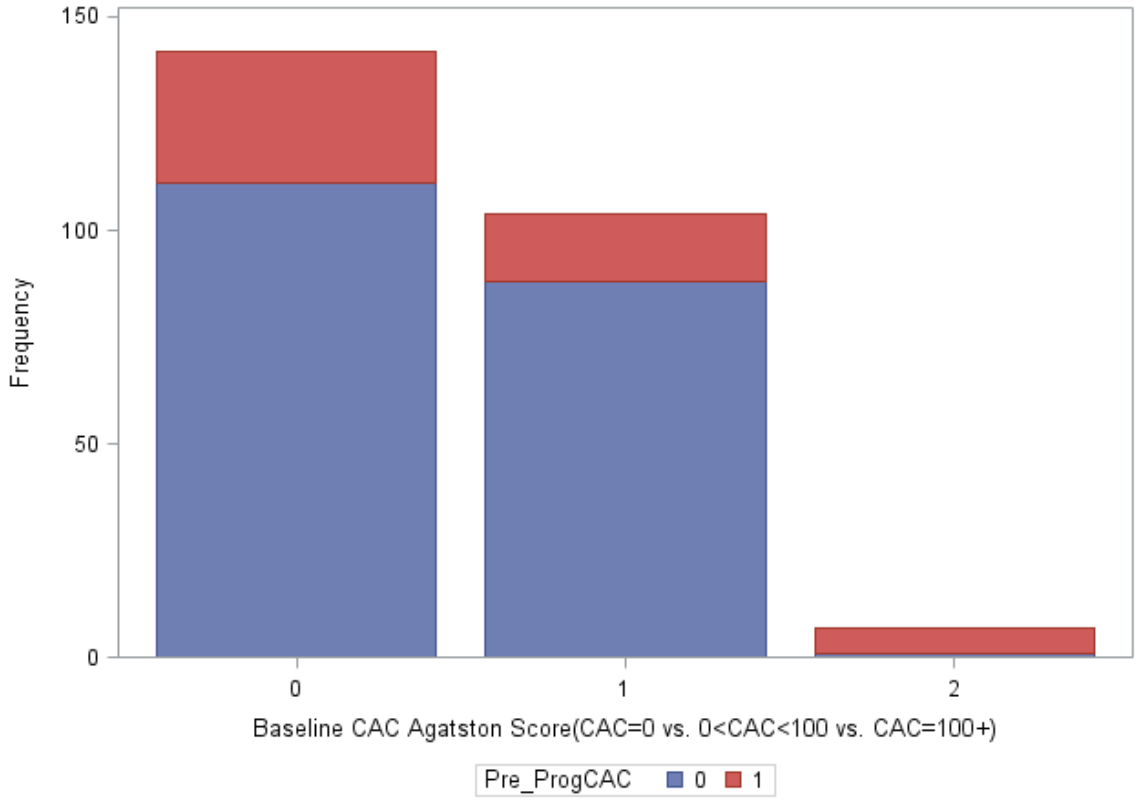


Figure 5.3 Percentage of women with CAC progression by baseline CAC Agatston score

6.0 Summary and Health Significance

Current knowledge as it pertains to associations between air pollution and atherosclerosis risk has primarily been generated in the general population, and often using a cross-sectional design. Original research, presented here, utilized cross-sectional and longitudinal designs with repeated measures of several key covariates. Our studies targeted middle-aged women during the menopausal transition and middle-aged women with PCOS, a condition with unique metabolic and physiological profiles of potential CAC risk. According to the biologic theory presented here, these are both 'canary in the coalmine' subpopulations given their unique vulnerability to $PM_{2.5}$ effects. We are using these studies to essentially capture a long-term pathophysiologic effect as an acute effect within a narrow and critical timeframe. In this regard, we helped to fill a gap in our knowledge of air pollution and CAC development and progression, as well as to point up the areas where further research is need.

In the first manuscript, we provide an epidemiological review of the current studies that evaluate the association between long-term $PM_{2.5}$ exposure and subclinical atherosclerosis measured by CAC. These studies were comprised of largely cross-sectional derived population based multiethnic groups of men and women, free of clinical CVD (MESA) (four studies), a cross-sectional study of the German HNRS by Hoffmann et al., one cross-sectional study used the CREATION Chinese cohort by Wang et al., as well as a study of Framingham offspring cohort by Doran et al., a cohort study involving offspring of the original study sample from the Framingham Heart Study in Massachusetts, U.S. We determined the extent to which these investigations included women and addressed covariates that related to their unique risk in their analyses. Based on seven investigations assessing the association between long-term $PM_{2.5}$ and CAC that published

between 2005 and 2020, as well as five studies evaluating the association between $PM_{2.5}$ and CIMT, we concluded that there is equivocal evidence with some, but not all studies reporting associations between $PM_{2.5}$ exposure and CAC presence or progression. In addition, gender was evaluated as an effect modifier in very few of those identified studies. For those that did evaluate gender further (e.g. Kaufman et al., 2016 the U.S. MESA study, Wang et al., 2019 the Chinese CREATION study), we found that subgroups of postmenopausal and older women had stronger associations between $PM_{2.5}$ exposure and CAC. Our review noted that there are differences with regard to the association between $PM_{2.5}$ and CAC among the all participants (men and women) when compared to those among women. Several previous studies such as WHI, SWAN and WISE have pointed out that visible increase in CVD risk seen after menopause and menopausal transition. These are not explained solely by chronologic aging. This review paper sharpened the focus of the dissertation project to disentangle chronologic age from the unique and specific physiologic changes occurring in middle-aged women.

The second manuscript evaluated the cross-sectional association between long-term $PM_{2.5}$ exposure and subclinical atherosclerosis, as measured by CAC, among midlife women of the CHARMIII study (N=301) 2001-2003. This is a case-control study, which included women with and without PCOS (136 PCOS cases and 165 controls) to determine CHD risk factors in a group of women with this reproductive and endocrinological condition. Within both groups, the annual mean of $PM_{2.5}$ levels were 16.6 ± 1.3 $\mu g/m^3$, which is similar exposure level as most of major U.S. cities, and we found no association between $PM_{2.5}$ and CAC presence. However, among women with PCOS a positive association was noted (OR=1.44; 95% CI: 1.02-2.04). No association was detected among women without PCOS. PCOS modified CAC risk affected by long-term $PM_{2.5}$ exposure (p for interaction=0.04).

In the third manuscript, we investigated the association between long-term $PM_{2.5}$ exposure and subclinical atherosclerosis, measured by CAC among midlife women from the Study of SWAN Heart study, an ancillary study to SWAN, 2001-2006. Among those women included (N=366, repeated measures of averagely 2.2 years apart), the annual mean of $PM_{2.5}$ levels were 16.5 ± 1.3 $\mu g/m^3$, and there was no overall association between $PM_{2.5}$ exposure and CAC presence, as well as presence of CAC progression. Within subgroups, there were positive, yet imprecise, point estimates. For example, the odds of higher CAC with higher exposure were slightly increased during the late peri-menopausal and natural postmenopausal status ($OR_{\text{presence of CAC progression}}$ =1.08, 95% CI: 0.74-1.58) or if women had exposure data available within 2 years FMP ($OR_{\text{repeated measures of CAC}}$ =1.13, 95% CI: 0.74-1.75). This might suggest that there is a temporal specificity of this association and both $PM_{2.5}$ and CAC should be measured accurately and within a narrow timeframe. In summary, there was no statistical evidence of association overall or within subgroups of the population. In evaluating effect modification by intermediate variables on the causal pathway, we observed stronger associations among women who were obese, among women with higher CRP and total cholesterol levels, and with lower HDL-C levels. This also argues for more consideration of the underlying biology and for the unique biology of mid-life transitions.

Taken together, our findings suggested that there is strong biologic rationale for an effect of $PM_{2.5}$ exposure on CAC presence and progression among middle-aged women. To date, there is no evidence of an overall association among all middle-aged women in both the SWAN heart and CHARMIII participants. This is consistent with previous reports summarized in our review paper. More work is needed to strengthen the methodologic framework for accurately measuring and estimating the acute effects of $PM_{2.5}$ exposure on CAC in a generally healthy population of middle-aged women. Nonetheless, in our two studies, support for this association is observed

among specific subpopulations of middle-aged women, e.g. women with PCOS (significant positive association) or women who were at late perimenopausal status (non-significant positive association). Null findings may be due to small sample size.

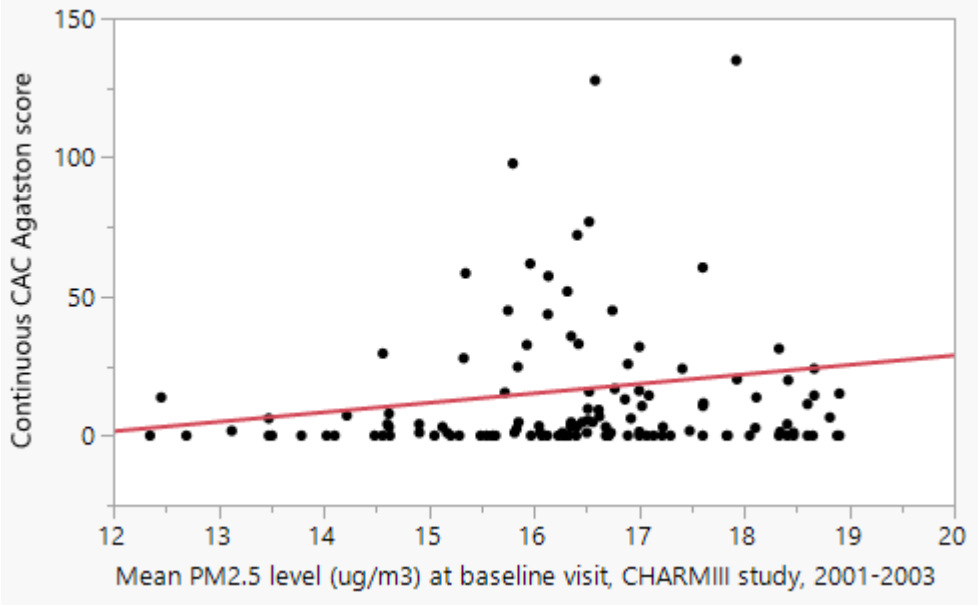
Early life exposures to $PM_{2.5}$ may be important in estimating causal effects over the life course. Air pollution exposure occurs over a lifetime and is known to have developmental effects (10,14,216). There are early life determinants of cardiovascular health (217). Early life determinants of both air pollution exposure and cardiovascular health were not explicitly accounted for in above studies. One example of this potential 'early life effect' is in our finding among PCOS cases. PCOS originated in early life and turned out to be the only subgroup of women in which there was a positive association of $PM_{2.5}$ and CAC.

The strongest outcome of this work is to establish a biologic theory and framework for future research on the acute effects of air pollution in mid-life women. Our findings argue for implementing highly accurate and longitudinal measures of $PM_{2.5}$ exposure and subclinical atherosclerosis, repeat sampling, attempts to assess early life determinants and underlying health, and modeling strategies that properly account for the complex biology underlying this association. A clinical concern with CAC is that there is a risk associated with ionizing radiation needed for the EBCT scan and repeat measures incur risk. Newer techniques such as high definition magnetic resonance imaging are needed and are being developed that should enhance this study in the future (218).

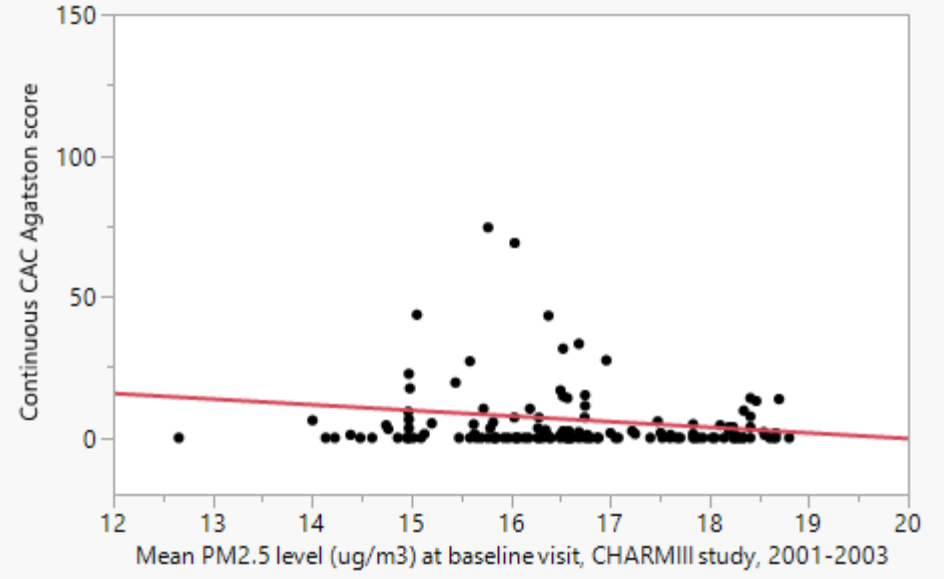
The public health significance of this work is to identify those vulnerable populations, so that we can reduce cardiovascular disease burden due to air pollution exposure among all people, and particularly among women later in life. Our findings were largely null due to limitations of our measures, small sample sizes, and unmeasured confounding by early life determinants. In our

study, mid-life women with PCOS had elevated odds of atherosclerosis with higher exposure to $PM_{2.5}$. Therefore, this study lays the groundwork for future study of adverse cardio-metabolic health changes during midlife due to adverse environmental exposure. If this relationship is proven to be true, this emphasizes the importance of controlling air pollution as another tool for preventing CVD.

Appendix A Materials for Chapter 4.0



Appendix Figure 1 Simple scatter with fit line of mean $PM_{2.5}$ ($\mu g/m^3$) at baseline visit 2001-2003 by continuous CAC, among women with PCOS



Appendix Figure 2 Simple scatter with fit line of mean $PM_{2.5}$ ($\mu g/m^3$) at baseline visit 2001-2003 by continuous CAC, among women without PCOS

Appendix Table 1 Effect modification of menopausal status on the association between $PM_{2.5}$ and CAC presence (<10 vs. ≥10), CHARMIII study (N= 246), 2001- 2003

Stratum in the interaction models	Total OR	p for interaction
Unadjusted model		
Reference	1.00	
Effect of interaction $PM_{2.5}$ & menopausal status	256.88	0.306
Effect of $PM_{2.5}$ in the interaction model	1.18	
Effect of Menopausal status in the interaction model	292.24	
Minimally adjusted model for Age, BMI, PCOS		
Reference	1.00	
Effect of interaction $PM_{2.5}$ & menopausal status	10305.16	0.159
Effect of $PM_{2.5}$ in the interaction model	1.17	
Effect of Menopausal status in the interaction model	>999.999	
Model adjusted for Age, BMI, Race, HRT, HDL, Ln (Trig), Glucose (Corrected), Insulin, and PCOS		
Reference	1.0000	
Effect of interaction $PM_{2.5}$ & menopausal status	25326.33	0.178
Effect of $PM_{2.5}$ in the interaction model	1.19	
Effect of Menopausal status in the interaction model	>999.999	

^ *246 out of 301 women had menopausal status data, with 191 were in premenopausal and 55 were in natural postmenopause at baseline of CHARMIII, 2001-2003.*

Appendix B Detailed Description of the Descriptive Statistics of Sociodemographic and Cardiovascular Risk Factors in Women with PCOS and Their Controls, by CAC Presence (≥10 vs. <10) (2001-2003)

Women with PCOS were younger than women without (46.7 vs. 49.0 years old), and were more likely to be Caucasian. There were no significant differences between PCOS cases and controls in the prevalence of current smoking (17.6% vs. 14.5%, p=0.465), current use of hormones (11.8% vs. 18.2%, p=0.118), education levels of college degree or above (77.2% vs. 72.1%, p=0.3), hypertension (33.1% vs. 26.1%, p=0.182) and impaired fasting glucose (23.5% vs. 17.6%, p=0.20) (Yes vs. No). Total cholesterol, LDL and HDL_2 were all lower for women with PCOS than controls, but the differences were not statistically significant (p=0.73, 0.47, 0.24, respectively). The fasting glucose was not significantly different between the women with and without PCOS (median: 86.0 vs. 91.0 mg/dL; Wilcoxon-Mann-Whitney test was conducted as the glucose level was not normally distributed, p= 0.2). (Table 4.1).

Within the group of women with PCOS, 34% had a CAC value greater or equal than 10 (Table 4.2), compared to 15% of women without PCOS had a CAC value ≥10 (Table 4.3). The PCOS cases with CAC≥10 had a mean age of 48 years old (SD=6.15), and those with CAC<10 had a mean age of 45.9 (SD=4.82) (p=0.03). The cases with a CAC≥10 had a much higher BMI of 37.0 (SD=6.8), compared to those with CAC<10 who had mean BMI of 29.0 kg/m^2 (SD=6.5) (p<0.0001). Twenty-four percent of those women with CAC≥ 10 were current smokers, compared with 14% of those with CAC<10 were current smokers (p>0.05). Among women with CAC≥10, 65% were occasional drinkers; and among women with CAC<10, 69% of them were drinker (p>0.05). A total of 34 PCOS women with CAC≥10 was in premenopausal or perimenopausal

(50%) and 24% had gone through natural post-menopause; and the remaining 26% of women had surgical menopause or other type of drug or radiation interventions rendering them postmenopausal. Among PCOS women with CAC<10, 81% were pre- or peri-menopausal and 10% were postmenopausal. The $PM_{2.5}$ annual mean levels were 17 $\mu g/m^3$ (SD=1.24), and 16.2 (SD=1.48), between the two CAC groups (CAC≥10 vs. CAC<10) of women with PCOS. (Table 4.2)

In bivariate analyses of women with PCOS by categorical CAC, BMI, waist and hip ratio, hypertension, percentage of natural postmenopausal, glucose and insulin levels were significantly greater in with higher CAC levels. Specifically, among those women with PCOS, those with higher CAC scores (CAC≥10) compared to lower CAC scores (<10) were more likely to be obese, to have higher waist and hip ratio, to have hypertension, were in natural postmenopausal status, and had higher levels of glucose and insulin. A comparable number of women reported current HRT use. The mean cholesterol levels, HDL2, LDL-C, and triglyceride levels were not significantly different between the two CAC groups. The subjects' remaining descriptive are shown in Table 4.2, for women with PCOS, stratified by categorical CAC (CAC <10, vs. CAC ≥10).

Within the group of women without PCOS, 14% had CAC ≥10 (Table 4.3). Among those women with CAC≥10, the mean age was 48 years old (SD=6.71) and mean BMI was 35 kg/m^2 (SD=7.19). Among women with CAC<10, their mean age was 49.1 (SD=5.64) and mean BMI was 27.3 (SD=5.1). Twenty-two percent of those women with CAC≥ 10 were current smokers, compared with 13% of them with CAC<10 were current smokers (p>0.05).

Among women with CAC≥10, 74% were occasional drinkers; and among women with CAC<10, 84% of them were drinker (p>0.05). A total of 19 women with CAC≥10 were in pre-, peri-menopausal (52%) or natural post-menopausal status (30%); and the other 18% of women

had surgical menopause or operations (e.g. radiation therapy, drug therapy); and among women with CAC<10, 58% of them were pre or peri-menopausal and 20% were postmenopausal. The mean annual $PM_{2.5}$ levels for the two CAC groups' women without PCOS were 16 $\mu g/m^3$ (SD=1.05), and 16.7 (SD=1.25) $\mu g/m^3$, for CAC≥10 vs. CAC<10 respectively. (Table 4.3)

In those bivariate analyses among the women without PCOS by categorical CAC, BMI, waist and hip ratio, impaired fasting glucose, type 2 diabetes, HDL2, triglycerides, glucose and insulin levels were all statistically significant different between the two CAC groups. Specifically, among those women without PCOS, those with higher CAC scores (≥10) compared to lower CAC scores (<10) were more likely to be obese, to have higher waist and hip ratio, to have type 2 diabetes and impaired fasting glucose, had lower level of HDL2, and had higher levels of triglycerides, glucose and insulin. Comparable number of women reported current HRT use. The mean cholesterol levels, LDL-C, and triglyceride levels had no statistically significant difference between the two CAC groups. Subjects' other descriptive sociodemographic and cardiovascular risk factors are presented in Table 4.3, for women without PCOS, stratified by categorical CAC (CAC <10, vs. CAC≥10).

Appendix C Materials for Chapter 5.0

Appendix Table 2 Characteristics of study participants at baseline by detectable CAC (=0 vs. >0) (N=366)

Characteristics	CAC=0, 2001-2004 N, %	CAC>0, 2001-2004 N, %	Chi-squared or Fisher' exact test p-value
All	194, 53%	172, 47%	
White	136, 70.1%	102, 59.3%	0.031
Smoker	27, 13.9%	21, 12.2%	0.570
Education Level			0.224
Less than college degree	25, 12.9%	32, 18.6%	
Some College/College Degree	107, 55.2%	82, 47.7%	
Post College	62, 32%	58, 33.7%	
Family Income (75,000+: Yes vs. No)	85, 43.8%	50, 29.1%	0.002
Ever used hormone therapy (Yes vs. No)	50, 25.8%	57, 33.1%	0.122
Menopause status ^^			0.409
Pre- and Early peri-menopausal	109, 56.2%	89, 51.7%	
Late peri-menopausal and Natural post	65, 33.5%	64, 37.2%	
CVD medication (Yes vs. No) ‡	32, 16.5%	51, 29.7%	0.003
Morbidity (Yes vs. No) *	37, 19.1%	74, 43%	<.0001
Categorical "Time to FMP" (Cutoff: 1 year) ‡‡			0.460
Pre: $PM_{2.5}$ exposure date to FMP date time interval <-1	72, 37.1%	59, 34.3%	
Mid: $-1 \leq PM_{2.5}$ exposure date to FMP date time interval ≤ 1	30, 15.5%	33, 19.2%	
Post: $PM_{2.5}$ exposure date to FMP date time interval>1	29, 14.9%	33, 19.2%	
Categorical "Time to FMP" (Cutoff: 2 years) **			0.647
Pre: $PM_{2.5}$ exposure date to FMP date time interval < -2	55, 28.4%	46, 26.7%	
Mid: $-2 \leq PM_{2.5}$ exposure date to FMP date time interval ≤ 2	56, 28.9%	56, 32.6%	
Post: $PM_{2.5}$ exposure date to FMP date time interval > 2	20, 10.3%	23, 13.4%	

Appendix Table 2 Continued

	Mean (SD)	Mean (SD)	p-value for T test or Wilcoxon-Mann-Whitney test*
$PM_{2.5}$ annual mean level ($\mu g/m^3$)	16.5 (1.3)	16.6(1.3)	0.708
Age at SWAN heart baseline visit (years)	50.9 (2.8)	51.7(2.7)	0.003
BMI (kg/m^2))	25.8 (3.9)	32.8(6.4)	<.0001
Waist (cm)	81.4 (9.2)	96.9(14.1)	<.0001
Physical activity total score†	8.2 (1.8)	7.5(1.6)	<.0001
Systolic blood pressure (SBP) (mmHg)	113.3 (14.9)	123(17.6)	<.0001
Diastolic blood pressure (DBP) (mmHg)	72.7 (9.7)	77.6(9.7)	<.0001
Cholesterol (mg/dL)	197.9 (33.8)	205.4(41.7)	0.061
High-density lipoprotein cholesterol (HDL-C) (mg/dL)	60.1 (14.6)	54.6(13.5)	0.000
Low-density lipoprotein cholesterol (LDL-C) (mg/dL)	116.9 (30.2)	123(35.6)	0.082
Triglyceride (mg/dL), **median (Q1-Q3)**	92 (71-121)	112.5 (83-163)	0.000
Follicle-stimulating hormone (FSH) (mIU/mL), **median (Q1-Q3)**	33.7 (15.3-87.7)	39.7 (16.3-74.1)	0.684
estradiol (E2) (pg/dL), **median (Q1-Q3)**	44.2 (16.3-99.6)	26.1 (15.6-51)	0.010
C-reactive protein (CRP) (ng/dL), **median (Q1-Q3)**	1.5 (0.6-3.5)	4.6 (1.6-10.6)	<.0001
HOMA, median (Q1-Q3)	1.6 (1.2-2.2)	2.7 (1.7-4.5)	<.0001
Plasminogen inhibitor-1 (PAI-1) (ng/dL), **median (Q1-Q3)**	10.4 (6.8-19)	18.9 (9.4-32.4)	<.0001
Tissue plasminogen activator Antigen (t-PA) (ng/dL), **median (Q1-Q3)**	6.2 (4.7-7.7)	8.1 (6-10.3)	<.0001

BMI, body mass index; CAC: coronary artery calcification; FMP, final menstrual period; FSH, follicle-stimulating hormone; and HOMA, homeostasis model assessment.

^^ Menopausal status had 39 women with missing data, as those women had surgical menopause, therefore, were coded into missing and excluded from the analyses related to menopause status

‡Taking medications for cholesterol, blood pressure, or diabetes mellitus

**Morbidity: history of hypertension and diabetes mellitus*

‡‡Time to FMP (cutoff: 1 year) had 110 women (30.1%) with missing data, as those women had no observed FMP

*** Time to FMP (cutoff: 2 years) had 110 women (30.1%) with missing data, as those women had no observed FMP*

†Modified Baecke Scores of habitual physical activity: higher scores indicating more physical activity

Appendix Table 3 Univariate regression: associations between baseline study variables and detectable CAC (CAC=0 vs. CAC>0) in women at Midlife (N=366)

Variables	Odds Ratio	95% Confidence Interval	p-value
Race (White vs Black)	1.61	1.04 - 2.48	0.031
Smoking (Yes vs. No)	0.84	0.45 - 1.55	0.570
Education Level (Ref. Less than College Degree)			0.227
Some College/College Degree	0.60	0.33 - 1.09	
Post College	0.73	0.39 - 1.38	
Family income (>= $75,000 vs. < $75,000)	0.50	0.32 - 0.78	0.002
Ever used hormone therapy (Yes vs. No)	1.43	0.91 - 2.24	0.123
Menopause status (Ref: Pre- and Early peri-menopausal)			
Late peri-menopausal & Natural Post	1.21	0.77 - 1.88	0.409
Categorical "Time to FMP" (Cutoff: 1 year)			0.461
Mid: $-1 \leq PM_{2.5}$ exposure date to FMP date time interval ≤ 1	1.34	0.74 - 2.45	
Post: $PM_{2.5}$ exposure date to FMP date time interval>1	1.39	0.76 - 2.55	
Categorical "Time to FMP" (Cutoff: 2 years)			0.648
Mid: $-2 \leq PM_{2.5}$ exposure date to FMP date time interval ≤ 2	1.20	0.70 - 2.05	
Post: $PM_{2.5}$ exposure date to FMP date time interval > 2	1.38	0.67 - 2.81	

Appendix Table 3 Continued

Time of $PM_{2.5}$ exposure to FMP	1.04	0.96 - 1.12	0.325
Age at SWAN heart baseline visit	1.12	1.04 - 1.21	0.004
Physical activity total score	0.78	0.68 - 0.88	<.0001
BMI	1.30	1.23 - 1.37	<.0001
Systolic blood pressure (SBP)	1.04	1.02 - 1.05	<.0001
Diastolic blood pressure (DBP)	1.05	1.03 - 1.08	<.0001
Cholesterol	1.01	1.00 - 1.01	0.063
High-density lipoprotein cholesterol (HDL-C)	0.97	0.96 - 0.99	0.000
Low-density lipoprotein cholesterol (LDL-C)	1.01	1.00 - 1.01	0.083
Ln (Triglyceride)	2.39	1.51 - 3.80	0.000
Ln (CRP)	1.84	1.54 - 2.21	<.0001
Ln (PAI-1)	1.75	1.37 - 2.24	<.0001
Ln (t-PA)	3.55	2.10 - 6.01	<.0001
Ln (E2)	0.79	0.65 - 0.96	0.016
Ln (FSH)	0.97	0.79 - 1.20	0.795
Ln (HOMA)	5.83	3.57 - 9.53	<.0001
CVD medication	2.13	1.29 - 3.52	0.003
Morbidity	3.20	2.01 - 5.12	<.0001

Appendix Table 3 Continued

BMI, body mass index; CAC: coronary artery calcification; FMP, final menstrual period; FSH, follicle-stimulating hormone; and HOMA, homeostasis model assessment.
Taking medications for cholesterol, blood pressure, or diabetes mellitus
Morbidity: history of hypertension and diabetes mellitus
Modified Baecke Scores of habitual physical activity: higher scores indicating more physical activity

Appendix Table 4 Univariate regression (for GLM): associations between study variables and repeated measurements of CAC in women at Midlife (N=619)

Variables	Odds Ratio	95% Confidence Interval	p-value
$PM_{2.5}$ annual mean	1.05	0.89 - 1.24	0.594
Race (White vs Black)	1.71	1.10 - 2.67	0.018
Family income (>= $75,000 vs. < $75,000)	0.50	0.32 - 0.78	0.003
Ever used hormone therapy (Yes vs. No)	1.29	0.81 - 2.04	0.287
Physical activity total score	0.76	0.67 - 0.86	<.0001
Menopause status (Ref: Pre-menopausal & Early peri)			
Late peri-menopausal & Natural post	1.17	0.76 - 1.79	0.449
Categorical "Time to FMP" (Cutoff: 1 year)			0.200
Mid: -1≤ $PM_{2.5}$ exposure date to FMP date time interval≤ 1	1.51	0.82 - 2.76	
Post: $PM_{2.5}$ exposure date to FMP date time interval>1	1.63	0.88 - 3.00	
Categorical "Time to FMP" (Cutoff: 2 years)			0.380
Mid: -1≤ $PM_{2.5}$ exposure date to FMP date time interval≤ 2	1.11	0.65 - 1.91	
Post: $PM_{2.5}$ exposure date to FMP date time interval>2	1.66	0.81 - 3.44	
CVD medication	2.07	1.24 - 3.43	0.005
Age at SWAN heart baseline and follow-up visit	1.13	1.04 - 1.22	0.003
BMI	1.30	1.23 - 1.36	<.0001

Appendix Table 4 Continued

Systolic blood pressure (SBP)	1.04	1.03 - 1.06	<.0001
High-density lipoprotein cholesterol (HDL-C)	0.97	0.95 - 0.98	<.0001
Ln (Triglyceride)	2.59	1.67 - 4.02	<.0001
Ln (CRP)	1.77	1.50 - 2.10	<.0001
Ln (t-PA)	2.67	1.73 - 4.11	<.0001
Ln (FSH)	0.88	0.71 - 1.09	0.252
Ln (E2)	0.85	0.70 - 1.03	0.100
Ln (HOMA)	6.99	4.44 - 11.01	<.0001

**Except $PM_{2.5}$, race, income, physical scores, medication use, and ever hormone use, all the above variables are time-varying repeated measures.*
BMI, body mass index; CAC: coronary artery calcification; FMP, final menstrual period; FSH, follicle-stimulating hormone; and HOMA, homeostasis model assessment.
Taking medications for cholesterol, blood pressure, or diabetes mellitus
Morbidity: history of hypertension and diabetes mellitus
Modified Baecke Scores of habitual physical activity: higher scores indicating more physical activity

Appendix Table 5 Univariate regression: associations between SWAN heart follow-up study variables and detectable CAC (CAC=0 vs. CAC>0) in women at Midlife (N=253)

Variables	Odds Ratio	95% Confidence Interval	p-value
Smoking (Yes vs. No)	1.09	0.50 - 2.40	0.832
Education Level			0.820
Some College/College Degree	1.02	0.51 - 2.05	
Post College	0.86	0.40 - 1.82	
Family income (>= $75,000 vs. < $75,000)	0.56	0.33 - 0.95	0.033
Ever used hormone therapy (Yes vs. No)	0.90	0.54 - 1.51	0.693
Menopause status (Ref: Pre- and Early peri-menopausal)			
Late peri-menopausal & Natural Post	1.13	0.65 - 1.98	0.668
$PM_{2.5}$ annual mean	1.07	0.87 - 1.32	0.530
Age at Visit	1.12	1.02 - 1.23	0.022
Physical activity total score	0.82	0.63 - 1.06	0.136
BMI	1.26	1.19 - 1.35	<.0001
Systolic blood pressure (SBP)	1.05	1.03 - 1.07	<.0001
High-density lipoprotein cholesterol (HDL-C)	0.95	0.93 - 0.98	<.0001
Ln (Triglyceride)	2.67	1.48 - 4.83	0.001

Appendix Table 5 Continued

Ln (CRP)	1.62	1.28 - 2.04	<.0001
Ln (E2)	1.04	0.78 - 1.39	0.778
Ln (HOMA)	11.56	5.34 - 25.06	<.0001
CVD medication	1.61	0.89 - 2.90	0.117
Morbidity	2.68	1.53 - 4.71	0.001
Day of cycle	0.92	0.44 - 1.89	0.811

BMI, body mass index; CAC: coronary artery calcification; FMP, final menstrual period; FSH, follicle-stimulating hormone; and HOMA, homeostasis model assessment.
Taking medications for cholesterol, blood pressure, or diabetes mellitus
Morbidity: history of hypertension and diabetes mellitus
Modified Baecke Scores of habitual physical activity: higher scores indicating more physical activit

Appendix Table 6 Effect of $PM_{2.5}$ on presence of CAC (<10 vs. ≥10) in the stratified groups (by exposure time to FMP (cutoff 1))

	Pre: > 1 y Before FMP		Mid: Within 1 y of FMP		Post: > 1 y After FMP		p for interaction
	N	OR (95% CI)	N	OR (95% CI)	N	OR (95% CI)	
Per 1 μg/m^3 increase in $PM_{2.5}$	88	0.77 (0.34 - 1.76)	51	1.64 (0.83 - 3.25)	55	0.55 (0.24 - 1.24)	0.186

** The above subgroup analyses were conducted using the final selected model, model 5($PM_{2.5}$ and presence of CAC), which adjusted for age, race, study site, imputed physical activity score, BMI, SBP, Ln (E2), Ln (CRP), Ln (HOMA).*

Appendix Table 7 Subgroup analyses on the association between $PM_{2.5}$ and presence of CAC (<10 vs. ≥10)

Per 1 μg/m^3 increase in $PM_{2.5}$			Presence of CAC (≥10)
	Stratum	N	OR (95% CI)
BMI	≤30 kg/m^2	190	1.05 (0.69 - 1.61)
	>30 kg/m^2	119	1.12 (0.81 - 1.54)
CRP	≤3 mg/L	170	1.13 (0.83 - 1.56)
	>3 mg/L	139	0.97 (0.68 - 1.40)
Race	White	203	1.05 (0.82 - 1.34)
	Black	106	1.05 (0.47 - 2.33)
Smoking	No	251	1.10 (0.85 - 1.43)
	Yes	43	0.79 (0.42 - 1.51)
Age	< 51 y	158	1.29 (0.79 - 2.11)
	≥ 51 y	151	1.02 (0.78 - 1.33)

**The above subgroup analyses were conducted using the final selected model, model 5($PM_{2.5}$ and presence of CAC), which adjusted for age, race, study site, imputed physical activity score, BMI, SBP, Ln (CRP), Ln (HOMA)*

Appendix Table 8 Sensitivity analyses on the association between $PM_{2.5}$ and presence of CAC (<10 vs. ≥10)

CAC presence (CAC≥10)		Without history of other diseases (hypertension and diabetes mellitus)
	N	OR (95% CI)
Per 1 μg/m^3 increase in $PM_{2.5}$	221	1.23 (0.91 - 1.68)

CAC presence (CAC≥10)		Without Taking medications (cholesterol, blood pressure, or diabetes mellitus)
	N	OR (95% CI)
Per 1 μg/m^3 increase in $PM_{2.5}$	238	1.12 (0.86 - 1.45)

CAC presence (CAC≥10)		Never hormone therapy user
	N	OR (95% CI)
Per 1 μg/m^3 increase in $PM_{2.5}$	212	0.93 (0.69 - 1.27)

**Model adjusted for age, race, study site, imputed physical activity score, BMI, SBP, Ln (CRP), Ln (HOMA)*

Appendix Table 9 Effect modifications of time since FMP on the association between $PM_{2.5}$ and presence of CAC (<10 vs. ≥10), among women with observed only FMP (n=212)

Stratum in the interaction model	Total Log Odds	Total OR	95% CI	p for interaction
Reference	0.00	1.00	Ref	
Effect of interaction term: $PM_{2.5}$ & Time to FMP	-0.01	0.99	0.90 - 1.08	
Effect of $PM_{2.5}$ in the interaction model	-0.10	0.91	0.66 - 1.24	
Effect of Time to FMP in the interaction model	0.17	1.19	0.26 - 5.45	
Effect of total effect of $PM_{2.5}$, time to FMP, and interaction $PM_{2.5}$ & Time to FMP	0.06	1.07	NA*	0.767

^Model adjusted for age, race, study site, imputed physical activity score, BMI, SBP, Ln (CRP), Ln (HOMA).
**Total OR was manually calculated by exponentiate the sum beta coefficient of $PM_{2.5}$, time to FMP, and interaction, and the 95% CI cannot be manually generated, therefore, not available.*

Appendix Table 10 Sensitivity analyses: effect modifications of time since FMP on the association between $PM_{2.5}$ and presence of CAC (<10 vs. ≥10), among women with imputed and observed FMP (N=309)

Stratum in the interaction model	Total Log Odds	Total OR	95% CI	p for interaction
Reference	0.00	1.00	Ref	
Effect of interaction term: $PM_{2.5}$ & Time to FMP	0.51	0.97	0.90 - 1.04	
Effect of $PM_{2.5}$ in the interaction model	0.04	1.04	0.82 - 1.31	
Effect of Time to FMP in the interaction model	0.51	1.67	0.49 - 5.69	
Effect of total effect of $PM_{2.5}$, time to FMP, and interaction $PM_{2.5}$ & Time to FMP	0.52	1.68	NA*	0.417

^Model adjusted for age, race, study site, imputed physical activity score, BMI, SBP, Ln (CRP), Ln (HOMA).
**Total OR was manually calculated by exponentiate the sum beta coefficient of $PM_{2.5}$, time to FMP, and interaction, and the 95% CI cannot be manually generated, therefore, not available.*

Appendix Table 11 Effect of $PM_{2.5}$ on presence of CAC progression in the stratified groups (by exposure time to FMP (cutoff 1))

Per 1 $\mu g/m^3$ increase in $PM_{2.5}$	Pre: > 1 y Before FMP		Mid: Within 1 y of FMP		Post: > 1 y After FMP		p for interaction
	N	OR (95% CI)	N	OR (95% CI)	N	OR (95% CI)	
	86	0.80 (0.47 - 1.34)	46	1.07 (0.64 - 1.80)	41	0.98 (0.53 - 1.81)	0.635

^ The above subgroup analyses were conducted using the final selected model, model 5($PM_{2.5}$ and presence of CAC progression), which adjusted for age, study site, race, BMI, total cholesterol.

Appendix Table 12 Subgroup analyses on the association between $PM_{2.5}$ and presence of CAC progression

Per 1 $\mu g/m^3$ increase in $PM_{2.5}$			Presence of CAC progression (Yes)
	Stratum	N	OR (95% CI)
BMI	≤30 kg/m^2	151	0.89 (0.59 - 1.34)
	>30 kg/m^2	94	1.12 (0.78 - 1.62)
CRP	≤3 mg/L	134	1.09 (0.74 - 1.61)
	>3 mg/L	105	1.10 (0.77 - 1.59)
Race	White	166	1.07 (0.81 - 1.42)
	Black	79	0.89 (0.41 - 1.90)
Smoking	No	204	1.11 (0.83 - 1.50)
	Yes	28	1.96 (0.47 - 8.23)
Age	<51 y	126	0.97 (0.61 - 1.55)
	≥ 51 y	119	1.07 (0.78 - 1.48)
Total cholesterol	< 200	132	0.61 (0.33 - 1.13)
	≥ 200	113	1.29 (0.95 - 1.74)
LDL	< 130	17	<0.001 (<0.001 - >999.999)
	≥ 130	228	1.07 (0.82 - 1.39)
HDL	< 40	157	1.22 (0.87 - 1.72)
	≥40	77	0.77 (0.46 - 1.29)

** The above subgroup analyses were conducted using the final selected model, model 5($PM_{2.5}$ and presence of CAC progression), which adjusted for age, study site, race, BMI, total cholesterol*

Appendix Table 13 Sensitivity analyses on the association between $PM_{2.5}$ and presence of CAC progression

Presence of CAC progression (Yes)		Without history of other diseases (hypertension and diabetes mellitus)
	N	OR (95% CI)
Per 1 $\mu g/m^3$ increase in $PM_{2.5}$	174	0.97 (0.68 - 1.40)

*Model adjusted for age, study site, race, BMI, total cholesterol

Presence of CAC progression (Yes)		Without Taking medications (cholesterol, blood pressure, or diabetes mellitus)
	N	OR (95% CI)
Per 1 $\mu g/m^3$ increase in $PM_{2.5}$	190	1.23 (0.90 - 1.67)

*Model adjusted for age, study site, race, BMI, total cholesterol

Presence of CAC progression (Yes)		Never hormone therapy user
	N	OR (95% CI)
Per 1 $\mu g/m^3$ increase in $PM_{2.5}$	171	1.15 (0.87 - 1.52)

**Model adjusted for age, study site, race, BMI, total cholesterol*

Appendix Table 14 Effect modifications of time since FMP on the association between $PM_{2.5}$ and presence of CAC progression, among women with observed only FMP (n=173)

Stratum in the interaction model	Total Log Odds	Total OR	95% CI	p for interaction
Reference	0.00	1.00	Ref	
Effect of interaction term: $PM_{2.5}$ & Time to FMP	0.02	1.03	0.95 - 1.11	
Effect of $PM_{2.5}$ in the interaction model	-0.10	0.90	0.66 - 1.23	
Effect of Time to FMP in the interaction model	-0.50	0.60	0.16 - 2.35	
Effect of total effect of $PM_{2.5}$, time to FMP, and interaction $PM_{2.5}$ & Time to FMP	-0.58	0.56	NA*	0.548

^Model adjusted for age, study site, race, BMI, total cholesterol
**Total OR was manually calculated by exponentiate the sum beta coefficient of $PM_{2.5}$, time to FMP, and interaction, and the 95% CI cannot be manually generated, therefore, not available.*

Appendix Table 15 Sensitivity analyses: effect modifications of time since FMP on the association between $PM_{2.5}$ and presence of CAC progression, among women with imputed and observed FMP (N=245)

Stratum in the interaction model	Total Log Odds	Total OR	95% CI	p for interaction
Reference	0.00	1.00	Ref	
Effect of interaction term: $PM_{2.5}$ & Time to FMP	0.03	1.03	0.95 - 1.11	
Effect of $PM_{2.5}$ in the interaction model	0.07	1.07	0.83 - 1.39	
Effect of Time to FMP in the interaction model	-0.52	0.59	0.17 - 2.10	
Effect of total effect of $PM_{2.5}$, time to FMP, and interaction $PM_{2.5}$ & Time to FMP	-0.43	0.65	NA*	0.462

^Model adjusted for age, study site, race, BMI, total cholesterol
**Total OR was manually calculated by exponentiate the sum beta coefficient of $PM_{2.5}$, time to FMP, and interaction, and the 95% CI cannot be manually generated, therefore, not available.*

Appendix Table 16 Effect of $PM_{2.5}$ on CAC repeated measures in the stratified groups (by exposure time to FMP (cutoff 1))

Per 1 $\mu g/m^3$ increase in $PM_{2.5}$	Pre: > 1 y Before FMP		Mid: Within 1 y of FMP		Post: > 1 y After FMP		p for interaction
	N	OR (95% CI)	N	OR (95% CI)	N	OR (95% CI)	
	203	0.84 (0.59 - 1.19)	104	1.02 (0.55 - 1.91)	98	0.60 (0.32 - 1.11)	0.557

** The above subgroup analyses were conducted using the final selected model, model 5($PM_{2.5}$ and CAC repeated measures), which adjusted for age, study site, race, and time varying: BMI, HDL, and SBP.*

Appendix Table 17 Association between $PM_{2.5}$ and detectable CAC (CAC=0 vs. >0)

Per 1 $\mu g/m^3$ increase in $PM_{2.5}$		Overall
	N	OR (95% CI)
Unadjusted model	366	1.03 (0.88 - 1.21)
Model 1	366	1.03 (0.88 - 1.20)
Model 2	331	1.06 (0.89 - 1.26)
Model 3	276	0.88 (0.70 - 1.12)
Model 4	251	0.89 (0.67 - 1.20)
Model 5	288	0.91 (0.73 - 1.13)

**Model 1: adjusted for site;*
Mode 2: model 1+age, race, study site, income;
Model 3: model 2+selected covariates (BMI, SBP, HDL, Ln (CRP), Ln (PAI-1), Ln (t-PA), total physical activity scores (imputed), Ln (Trig), ln (E2), Ln (HOMA), Day of cycle);
Model 4: model 3+menopause status, and hormone therapy use;
Model 5: adjusted for age, study site, race, income, BMI, total physical activity scores, SBP, Ln (CRP), Ln (HOMA).

Appendix Table 18 Effect of $PM_{2.5}$ on detectable CAC (CAC=0 vs. >0) in the stratified groups (by menopausal status; exposure time to FMP (cutoff 2 years)

Detectable CAC (CAC>0)	Pre- and Early peri-menopausal		Late peri-menopausal & Natural Post-menopausal		p for interaction
	N	OR (95% CI)	N	OR (95% CI)	
Per 1 μg/m^3 increase in $PM_{2.5}$	150	1.17 (0.83 - 1.65)	109	0.67 (0.43 - 1.04)	0.09

	Pre: Exposure to FMP time interval<-2		Mid: -2<=Exposure to FMP time interval<=2		Post: Exposure to FMP time interval>2		p for interaction
	N	OR (95% CI)	N	OR (95% CI)	N	OR (95% CI)	
Per 1 μg/m^3 increase in $PM_{2.5}$	77	0.97 (0.55 - 1.70)	85	1.26 (0.79 - 2.03)	36	<0.001 (<0.001 - 4.65)	0.100

** The above stratified analyses were conducted using the final selected model, model 5($PM_{2.5}$ and detectable CAC), which adjusted for age, study site, race, income, BMI, total physical activity scores, SBP, Ln (CRP), Ln (HOMA)*

For subgroup of postmenopausal women, additional variable, hormones therapy ever use, also was included in the model.

Appendix Table 19 Effect of $PM_{2.5}$ on detectable CAC (CAC=0 vs. >0) in the stratified groups (by exposure time to FMP (cutoff 1 year)

Per 1 μg/m^3 increase in $PM_{2.5}$	Pre: Exposure to FMP time interval<-1		Mid: -1<=Exposure to FMP time interval<=1		Post: Exposure to FMP time interval>1		p for interaction
	N	OR (95% CI)	N	OR (95% CI)	N	OR (95% CI)	
	99	1.13 (0.77 - 1.66)	46	1.08 (0.58 - 1.99)	53	0.38 (0.16 - 0.91)	0.141

** The above subgroup analyses were conducted using the final selected model, model 5($PM_{2.5}$ and detectable CAC), which adjusted for age, study site, race, income, BMI, total physical activity scores, SBP, Ln (CRP), Ln (HOMA).*

Appendix Table 20 Subgroup analyses on the association between $PM_{2.5}$ and detectable CAC (CAC=0 vs. >0)

		N	OR (95% CI)
BMI	≤30 kg/m^2	176	1.01 (0.76 - 1.34)
	>30 kg/m^2	112	0.75 (0.49 - 1.15)
CRP	≤3 mg/L	158	1.09 (0.82 - 1.45)
	>3 mg/L	130	0.71 (0.49 - 1.03)
Race	White	194	0.93 (0.74 - 1.18)
	Black	94	1.96 (0.79 - 4.86)
Smoking	No	245	0.98 (0.77 - 1.24)
	Yes	42	0.64 (0.35 - 1.16)
Age	<51 y	144	1.01 (0.71 - 1.46)
	≥ 51 y	144	0.84 (0.64 - 1.11)

** The above subgroup analyses were conducted using the final selected model, model 5($PM_{2.5}$ and detectable CAC), which adjusted for age, study site, race, income, BMI, total physical activity scores, SBP, Ln (CRP), Ln (HOMA)*

Appendix Table 21 Sensitivity analyses on the association between $PM_{2.5}$ and detectable CAC (CAC=0 vs. >0)

Detectable CAC (CAC>0)		Without history of other diseases (hypertension and diabetes mellitus)
	N	OR (95% CI)
Per 1 μg/m^3 increase in $PM_{2.5}$	197	1.00 (0.75 - 1.32)

		Without Taking medications (cholesterol, blood pressure, or diabetes mellitus)
	N	OR (95% CI)
Per 1 μg/m^3 increase in $PM_{2.5}$	222	0.99 (0.77 - 1.27)

		Never hormone therapy user
	N	OR (95% CI)
Per 1 μg/m^3 increase in $PM_{2.5}$	198	0.93 (0.71 - 1.22)

** Model adjusted for age, study site, race, income, BMI, total physical activity scores, SBP, Ln (CRP), Ln (HOMA)*

Appendix Table 22 Effect modifications of time since FMP on the association between $PM_{2.5}$ and detectable CAC (CAC=0 vs. >0), among women with observed only FMP (N=198)

Stratum in the interaction model	Total Log Odds	Total OR	95% CI	p for interaction
Reference	0.00	1.00	Ref	
Effect of interaction term: $PM_{2.5}$ & Time to FMP	-0.04	0.96	0.91 - 1.03	
Effect of $PM_{2.5}$ in the interaction model	-0.17	0.84	0.64 - 1.11	
Effect of Time to FMP in the interaction model	0.55	1.74	0.61 - 4.98	
Effect of total effect of $PM_{2.5}$, time to FMP, and interaction $PM_{2.5}$ & Time to FMP	0.34	1.41	NA*	0.247

^Model adjusted for age, study site, race, income, BMI, total physical activity scores, SBP, Ln (CRP), Ln (HOMA)
**Total OR was manually calculated by exponentiate the sum beta coefficient of $PM_{2.5}$, time to FMP, and interaction, and the 95% CI cannot be manually generated, therefore, not available.*

Appendix Table 23 Sensitivity analyses: effect modifications of time since FMP on the association between $PM_{2.5}$ and detectable CAC (CAC=0 vs. >0), among women with imputed and observed FMP (N=288)

Stratum in the interaction model	Total Log Odds	Total OR	95% CI	p for interaction
Reference	0.00	1.00	Ref	
Effect of interaction term: $PM_{2.5}$ & Time to FMP	-0.05	0.95	0.89 - 1.01	
Effect of $PM_{2.5}$ in the interaction model	-0.14	0.87	0.69 - 1.09	
Effect of Time to FMP in the interaction model	0.84	2.33	0.86 - 6.31	
Effect of total effect of $PM_{2.5}$, time to FMP, and interaction $PM_{2.5}$ & Time to FMP	0.65	1.91	NA*	0.078

^Model adjusted for age, study site, race, income, BMI, total physical activity scores, SBP, Ln (CRP), Ln (HOMA)
**Total OR was manually calculated by exponentiate the sum beta coefficient of $PM_{2.5}$, time to FMP, and interaction, and the 95% CI cannot be manually generated, therefore, not available.*

Appendix D Summary of the FMP Imputation Algorithm Used in Chapter 5.0

- FMP dates imputation for women missing their FMP dates was done within the full SWAN cohort (n=3302).
- For women who had 12 consecutive months of amenorrhea without taking hormone therapy (HT), FMP date was reliably assigned as the date of the last menstrual period (n=1804).
- For the rest of SWAN cohort (n=1498), FMP date was not assigned because women missed several visits, dropped out, had hysterectomy, had bilateral salpingo-oophorectomy, or received HT
- For our study (Chapter 4), all the included women (n=366) had either imputed FMP (n=110) or observed FMP (n=256) available.
- Analyses of complete data (excluding those with missing FMP date) may produce biased estimates and the results may not be applicable to the community sample represented by the SWAN cohort. Therefore, not to mention the increase in sample size, the advantages of reducing bias by including both women with observed and imputed FMP dates outweigh errors due to imputing data (Little and Rubin, 2019) (213).
- The software used for FMP dates imputation was IVEware (https://www.src.isr.umich.edu/wp-content/uploads/iveware-manual-Version-0.3.pdf), a free add-on set of routines for SAS from the University of Michigan. The software uses multivariate sequential regression, also known as Chained Equations; see the above link for additional information. The assumption was that the FMP data are "missing at random" conditional on the observed characteristics.
- The software imputed 10 FMP sets for women missing the FMP. To account for the uncertainty in the imputation process, our final estimates incorporated within and between imputation

variability as recommended by (Little and Rubin, 2019) (213). The utilized approach essentially taking a sample of size 10 from a woman estimated distribution of FMP date, and incorporating that distribution's variability (imputation uncertainty) in analyses accordingly.

- To improve the accuracy of FMP date imputation, the software bases the fill-in values on a participant's own available partial data of menstrual calendar by assigning woman-specific right and left endpoints for the FMP date imputation.
- Covariates included in imputation model were based on the literature, including papers from SWAN, and they are: demographics/SES, reproductive factors, lifestyle and other factors. The detailed list of those variables are included in a previously published paper (219).

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