QUANTITY AND QUALITY OF CARDIOVASCULAR FAT IN WOMEN AT MIDLIFE: ASSOCIATIONS WITH VARIOUS MARKERS OF CARDIOVASCULAR DISEASE RISK

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ABSTRACT

Background: Cardiovascular fat (CF) is a complex metabolically active organ and a cardiovascular disease (CVD) risk factor. Postmenopausal women have more CF compared to premenopausal women that may partly contribute to their increased risk of CVD.

Objectives: Our objectives were to determine whether CF quantities differed by race; whether CF quantities were associated with adipokines and coronary artery calcification (CAC) progression; and whether the quality of CF depots, measured via radiodensity, were associated with CVD risk measures in women at midlife.

Methods: We evaluated participants from the SWAN Cardiovascular Fat Ancillary Study (n=562 midlife women; mean age 50.9 ± 2.9 years; 62% White; 38% Black) who had cross-sectional measures (volumes and/or radiodensity) of CF depots (epicardial fat (EAT), paracardial fat (PAT), total heart fat (TAT), and aortic perivascular fat (PVAT)). Sample sizes varied for each study aim based on applied exclusion criteria (range 524 to 222). Multivariable linear or logistic regression models were used for analyses.

Results: Whites had higher quantities of CF for all depots compared to Blacks, independent of cardiovascular risk factors and abdominal visceral fat (VAT). Race modified the associations between adiposity measures and CF quantities such that Whites had more PAT for higher levels of BMI than Blacks; whereas, Blacks had more EAT for higher levels of VAT than Whites.

PAT was positively associated with leptin independent of cardiovascular risk factors and VAT, with stronger associations among Whites compared with Blacks. Lastly, we found that lower TAT radiodensity (poorer quality) was associated with a less favorable cardiometabolic profile and women with mid-range radiodensity values had significantly lower odds of CAC presence compared to low radiodensity values, independent of cardiovascular risk factors and BMI.

Conclusions: These analyses contribute to public health significance by enhancing our understanding of potential contributions of the quantity and quality of separate CF depots to CVD risk in midlife women. We found that the quantity of the mostly overlooked PAT depot may be especially important among midlife women with independent associations with leptin. Future studies should evaluate CF depots separately and further explore CF radiodensity as a marker of fat quality.

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1.0 INTRODUCTION

With nearly 70% of the U.S. population considered to be overweight or obese, the health effects of adiposity, especially in relation to cardiovascular disease (CVD), have received significant attention over the past ten years.[1] Even with the recent attention given to the topic, the exact nature of the association between excess adipose tissue and CVD remains to be clearly defined. Body mass index (BMI), the traditional measure of obesity, is not adequate in fully explaining the relationship between excess fat and CVD.[2-4] Adipose tissue is considered to be a metabolically active organ with potential endocrine and paracrine influences in the body.[5,6] Due to the heterogeneity of adipose tissue, research studies have focused on understanding how the location, quantity, and quality of fat depots individually affect metabolic and cardiovascular risk.[7-9]

It has been established that the CVD risk associated with excess adipose tissue varies depending on the location and quantity of the fat depot.[4,7] Though the exact process of adipose tissue distribution and accumulation is not definitively understood, one common theory states that in times of excess energy, fat accumulates in subcutaneous depots found below the skin.[7] Once this depot exceeds its capacity for expansion, fat begins to accumulate in areas and within cells not typically utilized for fat storage.[7] This accumulation may cause fat depots that generally contain minimal fat, such as cardiovascular fat depots around the heart and aorta, to expand and become dysfunctional.[10-12] The quantities of cardiovascular fat depots have been

shown to predict subclinical atherosclerosis and coronary artery disease (CAD), independent of BMI and visceral fat (VAT).[10,13,14]

Even though studies have shown an association between cardiovascular fat and CVD risk, very limited information is available on the factors that influence the quantity of these fat depots.[13-15] Certain adipose tissue depots, such as VAT and abdominal subcutaneous adipose tissue (SAT) have been shown to vary by gender, race, and age, indicating the possibility that cardiovascular fat depots may be influenced by these factors as well.[8,15,16] Interestingly, among middle-aged men, racial and ethnic differences exist in both the quantity of cardiovascular fat and in the magnitude of the associations between cardiovascular fat depots and other measures of adiposity, such as BMI and VAT.[15] These relationships have not been evaluated among midlife women who experience biological and physiological changes at midlife that may increase their risk of CVD over time.[17,18] Understanding the relationships between cardiovascular fat and demographics, as well as other adiposity measures in midlife women may help to identify critical areas for intervention.

The manner in which cardiovascular fat influences cardiovascular risk remains unclear; however, it is possible that these fat depots influence the local vasculature through the secretion of bioactive substances such as adiponectin and leptin, and may cause an inflammatory response resulting in increased levels of c-reactive protein (CRP).[19-22] The associations between adipokines and cardiovascular risk have been inconsistent and are not well understood. Low levels of adiponectin have been shown to correlate with insulin resistance and atherosclerosis.[19,23,24] Some evidence suggests that leptin has been associated with congestive heart failure and hypertension.[25] Some studies have shown that CRP levels are associated with

cardiovascular events, mortality, and may help to improve cardiovascular risk classification when added to traditional factors; however, the results have been inconsistent.[26-28]

Several studies have found that Black women tend to have a less favorable adipokine/cytokine profile with higher levels of leptin, CRP, and lower levels of adiponectin.[29,30] This is especially interesting since Black women tend to have a more favorable fat distribution with less VAT and less epicardial fat (EAT) compared with White women.[31-33] It has been hypothesized that the less favorable adipokine and cytokine profile seen among Black women may be a contributing factor to racial differences in cardiovascular risk.[29] Due to the complex heterogeneity and metabolic activity of adipose tissue, it is possible that the associations between cardiovascular fat quantity and CVD may be partially explained by levels of substances that these depots secrete and the inflammatory response adipokines incite; however, limited data is available on assessing the role adipokines play in the associations between cardiovascular fat and subclinical atherosclerosis.[7,8,34-36]

In addition to assessing the quantity of cardiovascular fat, measures used to assess the quality of fat may be important to consider when assessing the cardiovascular risk of cardiovascular fat.[9,37] Adipose tissue quality characteristics, such as adipocyte hypertrophy and hyperplasia, adipocyte hypoxia, macrophage accumulation, capillary density, and type of adipocytes have been evaluated, and evidence suggests associations between these adipose tissue quality parameters and CVD risk.[38-43] However, limited research is available due to the invasive nature of the procedures used to assess these fat quality characteristics.[38,39,42] Several recent studies have shown that computed tomography (CT) imaging is an effective method of measuring the quantity of fat by differentiating tissue types via the Hounsfield unit (HU) scale.[14] The HU scale represents the radiodensity of tissues in the body and is based on a linear

conversion of tissue attenuation measured by pixels.[44] In addition to using the HU scale to quantify fat, it has also been proposed as a novel way to assess fat quality.[9,45] Higher HU (less negative values) may indicate adipocytes that are densely packed with mitochondria and multiple lipid droplets, higher levels of vascularization and innervation, and fewer hypertrophic adipocytes.[9,46,47] High levels of lipid content in fat caused by hypertrophic adipocytes can increase free fatty acids that are associated with insulin resistance and endothelial dysfunction.[9,48,49] Therefore, higher fat HU values may be protective and represent a higher quality fat, while lower fat HU values (more negative values) may be more harmful and represent a lower quality fat.[9,46,47]

Only a few studies have evaluated the associations between fat HU values and CVD risk and the results have been inconsistent.[9,37,45,50] To date, no studies have evaluated the qualities of total heart fat (TAT) and aortic perivascular fat (PVAT), measured via HU, and their associations with CVD risk factors or subclinical measures of atherosclerosis, including coronary artery calcification (CAC) and thoracic aortic calcification (AC) in any population. By evaluating the associations between cardiovascular fat radiodensity and CAC and AC, we will assess and highlight the effects of a surrogate marker of fat quality on subclinical atherosclerosis in women at midlife.

This research work resulted in three manuscripts aimed at evaluating the associations between cardiovascular fat volume (paracardial fat (PAT), EAT, TAT, and PVAT) and adiposity measures (BMI, SAT, and VAT) and whether these associations differed by race; determining whether adipokines and cytokines explained the potential associations between cardiovascular fat and subclinical atherosclerosis; and assessing the relationships between cardiovascular fat

radiodensity and subclinical atherosclerosis, among midlife women. The specific aims for these manuscripts are defined in the following section.

2.0 SPECIFIC AIMS

The Study of Women's Health Across the Nation (SWAN) is a multicenter, community-based prospective study of women transitioning through menopause. The SWAN Heart Study was conducted at the Pittsburgh and Chicago study sites to evaluate subclinical atherosclerosis among healthy White and Black women. We utilized data from the SWAN Cardiovascular Fat Ancillary Study which was designed to quantify cardiovascular fat among SWAN Heart Study participants to assess the following specific aims of the three manuscripts for this dissertation.

Specific Aims for Manuscript 1: Determine whether race, overall adiposity, and central adiposity are associated with the quantity of cardiovascular fat depots (EAT, PAT, TAT, and PVAT; separate models) in cross-sectional analyses and evaluate whether associations between adiposity measures (BMI, VAT, and SAT; separate models) and volumes of cardiovascular fat depots vary by race in midlife women.

Hypothesis 1*: Cardiovascular fat volumes will differ by race. Black women will have lower volumes of cardiovascular fat compared to White midlife women.*

Hypothesis 2*: Cardiovascular fat volumes will be positively associated with BMI, VAT, and SAT and these associations will differ by race. Associations between VAT and*

volumes of cardiovascular fat depots will be stronger in Black compared to White midlife women.

Specific Aims for Manuscript 2: 1) Evaluate the associations between cardiovascular fat volumes (EAT, PAT, and TAT; separate models) and adipokine/inflammatory marker levels (leptin, adiponectin, leptin to adiponectin ratio (LA ratio), and CRP; separate models) independent of SAT or VAT, and assess the effect modification of race on these associations; 2) Determine whether baseline volumes of cardiovascular fat depots are associated with the presence and extent of CAC progression; and 3) Assess whether adipokine/inflammatory marker levels (leptin, adiponectin, LA ratio, and CRP; separate models) may explain the associations between baseline cardiovascular fat volumes and the presence and extent of CAC progression.

Hypothesis 1*: Among midlife women, higher EAT, PAT, and TAT volumes will be associated with lower levels of adiponectin and higher levels of leptin, LA ratio, and CRP, independent of SAT or VAT.*

Hypothesis 2: *Race will modify the associations between cardiovascular fat depot volumes and adipokine/inflammatory marker levels with stronger associations among Black women than White women. These interactions will be independent of SAT or VAT.*

Hypothesis 3*: Higher baseline EAT, PAT, and TAT volumes will be associated with a higher odds of the presence of CAC progression and a greater extent of CAC progression among women at midlife.*

Hypothesis 4*: Adipokine/inflammatory marker levels will help to explain the proposed associations between baseline cardiovascular fat volumes and the presence and extent of CAC progression among women at midlife.*

Specific Aims for Manuscript 3: Evaluate the cross-sectional associations between cardiovascular fat radiodensity values (radiodensity of TAT and PVAT; separate models) and CVD risk factors and subclinical atherosclerosis (CAC and AC) in women at midlife.

Hypothesis 1*: Lower TAT and PVAT radiodensity values will be associated with an adverse CVD risk profile.*

Hypothesis 2*: Lower TAT and PVAT radiodensity values will be associated with presence of CAC and AC independent of traditional CVD risk factors and other adiposity measures.*

3.0 BACKGROUND

The menopausal transition is a period of time in which women undergo significant biological, psychological, and societal changes.[51] Postmenopausal women tend to have an adverse adipose tissue distribution with higher volumes of VAT and cardiovascular fat when compared with premenopausal women.[52,53] Postmenopausal women are at an increased risk of CVD, which has a long subclinical component of development and is the leading cause of death among women.[17,18] Atherosclerotic calcification is a progressive condition that is similar to bone formation and is an indicator of the extent of atherosclerotic disease evolution.[54,55] CAC is the most studied manifestation of atherosclerosis and has been shown to predict CVD events.[56-58] Understanding characteristics and determinants of cardiovascular fat and how the volume and the quality of these fat depots may influence CVD risk factors and subclinical atherosclerosis may help to identify critical areas for intervention in women transitioning through menopause.

In the following chapters, cardiovascular fat pathophysiology, methods of measurement, potential determinants, and metabolic activity are described. Next CAC and AC pathophysiology and the methods of measurement and quantification are reviewed; followed by a description of the epidemiology of the prevalence and progression of calcification and novel risk factors for CAC and AC. Lastly the potential role of adipokines and cytokines in explaining the associations between cardiovascular fat and atherosclerotic calcification are reviewed.

3.1 CARDIOVASCULAR FAT

3.1.1 Pathophysiology of Cardiovascular Fat

The heart and nearly all arteries in the human body are surrounded by adipose tissue, termed cardiovascular fat, which up until recently was considered to be primarily for support and protection.[59,60] Although considerable variability exists in the definitions of the cardiovascular fat depots, the following cardiovascular fat definitions will be used in this dissertation: EAT is the fat within the pericardial sac; PAT is the fat outside of the pericardial sac; TAT is the summation of EAT and PAT; PVAT is the fat along the descending aorta. Recent evidence has shown that these fat depots secrete numerous pro- and anti-inflammatory substances making them active endocrine and paracrine organs.[60-62] Some of the common adipokines released by these fat depots include adiponectin and leptin, which tend to have anti-inflammatory and pro-inflammatory actions, respectively.[63] In a physiological state, the release of these substances tend to counterbalance inflammation and may serve as a buffer against exposure to excessive levels of circulating fatty acids, thus serving to protect surrounding organs and vasculature.[61,62,64]

The current theory asserts that these fat depots become dysfunctional in states of excess adiposity.[4,61] In a physiological state, fat cells enlarge in response to excess energy.[65] This response signals the proliferation of new adipocytes from precursor cells.[65] When adipocyte hyperplasia is impaired, existing adipocytes continue to buffer fatty acids resulting in extreme hypertrophy, dysfunctional adipose tissue, and fat accumulation in ectopic fat depots.[4,65] As these hypertrophic adipocytes continue to enlarge, the increased distance from the vasculature and reduced capillary density can prevent the adipocytes from getting enough blood flow and oxygen resulting in hypo-perfusion and hypoxia.[65-67] Adipose tissue that is not receiving

enough oxygen and blood causes an inflammatory response and recruits macrophages in order to increase blood flow, stimulate angiogenesis, and clean out dead adipocytes.[41,67,68] Hypertrophic adipocytes have been shown to shift towards secreting predominately pro-inflammatory adipokines and cytokines and may incite increased levels of inflammatory markers such as CRP.[4,21]

It has been suggested that cardiovascular fat may play an important paracrine role in atherosclerosis on local arteries via an outside-to-inside manner.[61,69] Inflammatory substances could diffuse the arterial wall to interact with the adventitial, medial, and intimal layers of the artery or they may be released directly into the vasa vasorum and be transported into the arterial wall.[22,61,70,71] Therefore, it is important to assess the associations between specific cardiovascular fat depots and subclinical atherosclerosis in the arteries located near the specific fat depot.[22,61,70]

3.1.2 Measures of Cardiovascular Fat Quantity and Quality

Cardiovascular Fat Depots

The adipocytes that surround arteries are more heterogeneous and are morphologically and functionally different compared to adipocytes in other regional fat depots, which may reflect the overall quality of the adipose tissue.[61] Studies have found that the quality of fat varies between regional depot locations and even among different vascular beds.[61,72-74] Studies in humans and mice have shown that the fat surrounding the coronary artery and thoracic aorta share similarities with brown adipocytes; while the fat surrounding the abdominal aorta closely resembles white adipocytes.[72,74,75] Therefore, due to cardiovascular fat differences that may be dependent on anatomical location, it is important to consider cardiovascular fat depots separately.

In the current literature, significant variability exists between studies on how cardiovascular fat depots were defined and how they were measured. For the purpose of this dissertation, EAT is defined as the fat located within the visceral pericardium with no muscle fascia dividing it from the myocardium, PAT is the fat outside of the pericardial sac, and TAT is considered to be the summation of EAT and PAT.[52] PVAT is the adipose tissue along the descending aorta which does not include the ascending aorta or the aortic arch.[52] Due to the significant definition differences between studies referenced in this dissertation, the terminology of EAT, PAT, and TAT have been standardized to represent the definitions explained above. Figure 3.1 illustrates the different fat depots measured using a CT image. The pericardium is traced in yellow. EAT is highlighted in red, PAT is highlighted in blue, TAT is the combination

of the blue and red areas, and PVAT section is traced in green and highlighted in pink. The anatomical borders and methods of quantification are described in the following section.

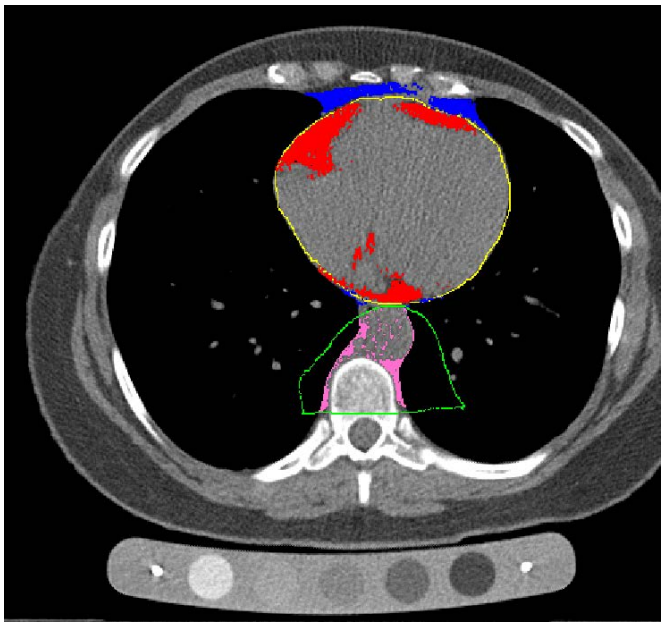


Figure 3-1: Cardiovascular fat depots

Methods of Measurements

Echocardiography, CT, and magnetic resonance imaging (MRI) are the three main types of imaging modalities used for quantifying cardiovascular fat.[76] Each method has advantages and disadvantages in regards to image quality, radiation exposure, expense, and patient comfort. Early stages of assessing EAT were typically performed using echocardiography due to the ease, availability, and minimal radiation exposure.[76,77] Also, it is often used for other clinical reasons for high risk cardiac patients and measuring adipose tissue incurred no extra cost.[76,77] In spite of these advantages, this method has several significant disadvantages that, in general, may make it

the least desirable method of assessment.[76,78] Echocardiographic measurements are based on a single point, typically on the right ventricular free wall, which provides a 2D thickness measurement.[76,77] EAT is not evenly distributed around the heart with significant variability between men and women; therefore, only assessing the adipose tissue in one location may provide a misleading quantity of fat depending on the individual's fat distribution.[76,77,79] Lastly, this method is not able to assess other types of perivascular fat and obesity may limit the image quality.[76]

In contrast, MRI is the gold standard of measurement for cardiovascular fat and ensures no radiation hazards to the patient.[76] However, this method is the least used method to assess cardiovascular fat because it is the most expensive of the three imaging modalities, is more time-consuming, and is not as tolerated by patients.[76] Today, CT is the most commonly used method of assessing these fat depots because it provides a nice balance between echocardiography and MRI and it is used for the purpose of measuring CAC.[76] This method is easy to perform, allows volumetric assessments with good reproducibility of multiple depots, and can simultaneously assess atherosclerotic calcification.[76] The disadvantages are that it is more expensive than echocardiography and there is some radiation exposure to the participants.[76] Computed tomography is often used to assess CA; therefore, cardiovascular fat can be readily quantified using external software without extra scanning.[80] The two main types of CT scanners, multidetector computed tomography (MDCT) and electron beam computed tomography (EBCT), are comparable and provide images with high temporal and spatial resolutions.[80,81] For the purpose of this dissertation, EBCT was utilized to assess cardiovascular fat depots.

Adipose tissue depots are determined by identifying anatomical landmarks and by quantifying the fat within these boundaries.[76] Adipose tissue is identified on CT images because

of its low attenuation measured via HU.[76] Semiautomatic external software is then used to quantify the volume of fat within each depot by setting a HU threshold.[76] The typical HU range for adipose tissue is -190 to -30; however, this range varies between studies with some investigators using a threshold of -195 to -45 HU.[35,82,83] In addition to serving as a means to distinguish adipose tissue from other tissues in the body, HU have been shown to possibly represent the quality of the fat.[9,45-47] Higher HU may indicate adipocytes that are densely packed with mitochondria and multiple lipid droplets, higher levels of vascularization and innervation, and fewer hypertrophic adipocytes.[9,46,47] Lower HU may indicate high levels of lipid content in fat caused by hypertrophic adipocytes, which can increase free fatty acids that are associated with insulin resistance and endothelial dysfunction.[9,48,49] Therefore, higher fat HU values may be protective and represent a higher quality of fat, while lower fat HU values may be more harmful and represent a lower quality fat.[9,46,47]

Quantifying EAT, PAT, and TAT

For all analyses in this dissertation, EAT, PAT, and TAT were measured using 3-mm-thick transverse images obtained by GE-Imatron C150 Electron Beam Tomography scanners (GE Medical Systems, South San Francisco, CA, USA) to quantify fat from 15 mm above to 30 mm below the superior extent of the left main coronary artery.[52] EAT was defined as the fat within the pericardial sac. The anterior border of the PAT volume was defined by the chest wall and the posterior border by the aorta and the bronchus.[52] Adipose tissue was distinguished from heart tissue by a threshold of -190 to -30 HU using volume analysis software (GE Healthcare, Waukesha, WI, USA).[52] All EAT, PAT, and TAT measurements were completed at the Los Angeles Biomedical Research Institute, Harbor-UCLA Medical Center, CA, USA. For EAT and

TAT measurements, readers manually traced the anatomical boundaries every 2-3 slices below the starting point and would allow the software to automatically trace the segments in between these manually drawn slices. PAT was calculated by subtracting EAT from TAT; therefore, only mean HU were calculated for EAT and TAT. Excellent within- and between-reader spearmen correlation coefficients of 0.97 have been reported for this fat quantification protocol.[52] A single mean HU value for each depot, expect PAT, was used to represent the overall quality of the adipose tissue.

Quantifying PVAT

For all analyses in this dissertation, PVAT was measured using 6-mm transverse images obtained by GE-Imatron C150 Electron Beam Tomography scanners (GE Medical Systems, South San Francisco, CA, USA) to quantify adipose tissue along the descending aorta. The pulmonary bifurcation served as the proximal boundary, while the image immediately above the first lumbar vertebra marked the distal boundary.[83] The proximal anterior border was the bronchus, which distally became the esophagus and then the crus of the diaphragm, while the vertebral body served as the posterior border. Readers at the University of Pittsburgh, Pittsburgh, PA, USA, used the Slice-O-Matic Software V4.3 (TomoVision, Magog, Qc, Canada) to manually draw every slice and fat was defined using a threshold of -190 to -30 HU.[83] This protocol has excellent intra-reader and inter-reader reproducibility (intraclass coefficients 0.999 and 0.998, respectively).[83] A single mean HU value was used to represent the overall PVAT quality.

3.1.3 Potential Determinants of Cardiovascular Fat

Age and Menopause

The aging process in both men and women has been associated with increased overall adiposity and regional adipose tissue distribution changes, including increases in cardiovascular fat accumulation.[84,85] It has been hypothesized that as age increases, subcutaneous adipocytes are less able to store lipids and buffer circulating free fatty acids leading to increases in fat accumulation in visceral depots, such as around the heart and vasculature.[85,86] Some studies suggest that hormonal changes in women caused by ovarian aging during the menopausal transition may contribute to adipose tissue distribution independent of chronological age.[52,87] Among SWAN Cardiovascular Fat Study participants, late peri- and postmenopausal women had higher EAT, PAT, and TAT volumes, independent of age, obesity, physical activity, smoking, alcohol consumption, medication use, and comorbidities.[52] Interestingly, although higher PAT and TAT volumes were associated with lower levels of estradiol, changes in estradiol were only associated with the PAT depot.[52] Women with the greatest decline of estradiol after 4 years had higher PAT volumes compared to women who experienced the least decline in estradiol, independent of the covariates listed above.[52]

Adiposity Measures and Race

Based on the theory that excess cardiovascular fat accumulation occurs when the capacity of depots designed to store excess fat exceeds their limit, it would be expected that increases in other adiposity measures, especially VAT, could be an indication of increases in cardiovascular fat.[15,86,88] Although studies have shown that cardiovascular fat is positively correlated with BMI; stronger associations have been found with waist circumference and VAT.[15,59,79,85]

Cardiovascular fat is a type of visceral fat and once excess fat accumulates in visceral areas, it may be more likely to accumulate in cardiovascular fat depots as well.[79,85]

Significant racial differences exist in fat distribution with Black men generally having lower volumes of cardiovascular fat and VAT, when compared to White men.[15,31,32,89] These racial differences among men remain significant after further adjustment for overall adiposity, with Black men having lower EAT, PAT, TAT, and VAT volumes compared to White men.[15,31] Most recently a study among midlife men showed that not only are there differences in cardiovascular fat volumes between Black and White men, but the magnitude of associations between BMI and cardiovascular fat varied by Black and White race as well.[15] With every one-increment increase in BMI, White men had more EAT and TAT, compared to Black men.[15]

Research assessing the racial differences in cardiovascular fat among women is limited. Evidence suggests that compared to White women, Black women have higher BMI levels and lower unadjusted EAT volumes.[31,32] It is unclear whether Black women have lower cardiovascular fat volumes independent of adiposity measures and whether race modifies the associations between adiposity measures and cardiovascular fat measures among women. Assessing how race modifies the effects of BMI and VAT on cardiovascular fat volumes may be especially important due to the regional adipose tissue distribution transformations seen in postmenopausal women.[52,87]

3.1.4 Cardiovascular Fat as a Source of Potential Adipokines and Cytokines

Adipose tissue is a metabolically active paracrine and endocrine organ that secretes many adipokines with significant influences on several bodily functions.[5,90] The roles of these bioactive substances are complex and often interrelated and have been shown to incite an inflammatory response in states of adipose tissue dysfunction.[63,91] During periods of caloric surplus, adipose tissue is designed to buffer the excess energy through adipocyte enlargement and recruitment.[65] When the fat depot is no longer able to recruit new adipocytes, the current adipocytes continue to expand and become hypertrophic.[65,92] These hypertrophic adipocytes reduce the amount of oxygen and blood to the fat depot causing cell death and macrophage recruitment.[4,65] In addition, the size of hypertrophic adipocytes have been shown to be directly associated with the increased production and secretion of pro-inflammatory adipokines and the reduction of anti-inflammatory adipokines.[92]

Because of this inflammatory response and the intricate functionality and secretory profile, it is possible that cardiovascular fat depots may be especially important due to the close proximity to the heart and vasculature.[91,93,94] It has been hypothesized that a crosstalk between cardiovascular fat and the heart and vasculature may occur with adipocyte secreted proteins and hormones directly migrating into the bordering cells or being released through the vasa vasorum.[22,95] Adiponectin and Leptin are two of the most prolific and commonly researched adipokines that are secreted directly from adipocytes.[63] Other adipocytokines, such as interleukin-6, have been shown to stimulate an inflammatory response resulting in increased CRP levels.[91] Although findings have been inconsistent for the associations between adiponectin, leptin, and CRP and cardiovascular risk, some studies have found correlations with

CAC; therefore, adiponectin, leptin, and CRP may be important factors relating cardiovascular fat to CVD.[96-99]

Adiponectin

Adiponectin is an adipocyte-derived protein that is expressed in adipose tissue and abundant in plasma.[100-102] Traditionally thought to be anti-inflammatory and protective, adiponectin levels are decreased in states of obesity with a stronger inverse association with VAT compared to SAT.[101,102] In addition, low levels of adiponectin correlate with insulin resistance and atherosclerosis.[23,24,63] It has been shown that adiponectin modulates vascular tone by increasing the bioavailability of nitric oxide; however, in states of obesity, this capacity is lost with resulting inflammation and oxidative stress.[12] Adiponectin displays anti-atherosclerotic properties by acting as an antithrombotic factor, reducing platelet aggregation, and inhibiting macrophage accumulation.[103-105] In addition, adiponectin has been shown to influence levels of inflammatory substances by inhibiting tumor necrosis factor expression and inducing the production of anti-inflammatory cytokines such as interleukin-10.[63,106]

In general, women tend to have higher adiponectin levels when compared to men; and White women have higher adiponectin levels compared to Black women.[107,108] Recent studies suggest that the protective effects of adiponectin may not be as clear cut as formerly thought, especially in higher risk populations, reaffirming the complexity of adipokines and cytokines.[109] Inconsistent with evidence showing adiponectin as protective, some studies have shown that higher adiponectin levels are associated with cardiovascular events and mortality among people with CAD or higher cardiovascular risk, alluding to the possibility of adiponectin

resistance.[109,110] More research is necessary to understand the mechanisms in which adiponectin levels influence CVD.

Leptin

Leptin is secreted primarily by adipocytes and is considered to be a pro-inflammatory adipokine.[63,100] The main role of leptin is to regulate body fat, hunger, and energy expenditure through the hypothalamus.[100,103] Theoretically, decreased leptin levels would increase appetite and lower energy expenditure by signaling to the brain that the body is starving; however, higher levels of leptin are often associated with obesity.[103,111] It has been hypothesized that in states of obesity defects occur in leptin receptor signaling or in the transport of leptin across the blood-brain barrier, resulting in a resistance to leptin in the body.[100,103,111] High leptin levels have been shown to be associated with increased production of inflammatory cytokines, increased cellular proliferation and oxidative stress, promotion of smooth muscle cell proliferation, and increased vascular calcification.[63,102,103,112] After adjusting for age and adiposity, serum leptin levels tend to be higher in women than in men; and higher in Black women compared to White women.[29,63]

C-Reactive Protein

As adipose tissue becomes dysfunctional, the balance of adipocytokine secretions is disrupted with an increase in the release of pro-inflammatory substances, resulting in a low-grade state of inflammation as seen in obesity.[4,91] CRP is an acute phase protein and a marker of inflammation which is primarily produced by hepatocytes and regulated by the adipocytokine, interleukin-6.[109,113,114] Interestingly, it has also been shown that adipocytes and coronary artery smooth muscle cells can synthesize or produce CRP under inflammatory stimuli.[109,114,115]

Although, CRP levels are sensitive to inflammatory stimuli, within-person CRP levels in a healthy population tend to be stable overtime.[116] CRP is well recognized as a useful tool to measure systemic inflammation.[116] Women tend to have higher CRP levels compared to men and Blacks tend to have higher levels compared to Whites.[117] Obesity has been shown to be correlated with increased CRP levels.[118,119] Elevated CRP has been linked to endothelial dysfunction by promoting the expression of adhesion molecules in endothelial cells, inhibiting nitric-oxide synthesis, increasing the number of foam cells, and increasing the release of tumor necrosis factor-α.[113,120] Overall the associations between CRP and cardiovascular risk have been inconsistent; however, some studies have found CRP to be associated with cardiovascular events and mortality, and CRP may help to improve the cardiovascular risk classification when added to traditional risk factors.[26-28]

With the increased risk of CVD seen among midlife women, it is important to understand the mechanisms behind this increased risk.[17,18] The regional adipose tissue changes, especially higher cardiovascular fat volumes, seen in postmenopausal women may be an important factor.[52] Understanding the metabolic activity and whether adipokines and inflammation explain the associations often seen between cardiovascular fat and subclinical atherosclerosis is an important area of research due to the limited data available among this population.

3.1.5 Cardiovascular Fat and Future Cardiovascular and Mortality Events

Some studies, but not all, have found that the quantity of cardiovascular fat predicts future coronary heart disease (CHD) events and all-cause mortality.[121] Among the Framingham Heart Study (n=3,089; 49% female; mean age, 50.2±10 years; maximum follow-up, 7.4 years), Britton et al. failed to find any associations of PVAT and EAT with incident CVD or all-cause mortality, after adjusting for cardiovascular risk factors and BMI.[122] However, among a subsample of the Multi-Ethnic Study of Atherosclerosis (MESA) participants (n=1,119; 63% female; mean age, 60±10 years; follow-up, 5 years); Ding et al. found that every 1 SD increment increase in EAT volume was associated with a 26% greater risk of incident CHD events after adjusting for several CVD risk factors and BMI (HR 1.26; 95% (CI 1.01, 1.59); $p<0.05$).[13] These results were attenuated after adjusting for waist circumference (HR 1.24; 95% CI (0.99, 1.57); $p>0.05$).[13] Interestingly, among a Japanese population with suspected CAD (n=722; 61% female; mean age, 65±11 years; mean follow-up, 4±2 years), Yamamoto et al. found when compared to low BMI and low EAT volume, only Japanese participants in the lowest quartile of BMI and with high EAT volume had a higher risk of major coronary events (HR 5.45; 95% CI (1.51, 25.3); p=0.009) and all coronary events (HR 3.91; 95% CI (1.30, 13.0); p=0.015) after adjusting for age, sex, and CAC score.[123] Participants within the 2nd, 3rd, and 4th quartiles of BMI did not have higher risk of any events, regardless of EAT volume, indicating that high EAT volumes may be important for high-risk Japanese men and women with normal BMI levels.[123]

However, a few studies did find stronger associations between cardiovascular fat and future events. Among participants in Heinz Nixdorf Recall Study (n=4,093; 53% female; mean age, 59±8 years; mean follow-up, 8±2 years), Mahabadi et al. found that for every doubling in

EAT volume the risk of experiencing a fatal or nonfatal coronary event increased significantly (HR 1.50; 95% CI (1.07, 2.11); p=0.02) after adjusting of age, sex, waist circumference, cardiovascular risk factors, and CAC.[121] Among participants of the Rancho Bernando Study (n=343; 55% female; mean age, 67±7 years; median follow-up, 12.6 years), Larsen et al. found that the highest tertile of EAT area ($\geq$194.3 cm^2) had a significantly higher risk of all-cause mortality (HR 2.62; 95% CI (1.06, 6.55); p=0.04) when compared to the lowest EAT area tertile (<126.5 cm^2) after adjusting for age, sex, cardiovascular risk factors, VAT, and adipocytokines.[124] Among a high-risk population with stable CAD (n=145; 35% female; mean age, 60±10 years; mean follow-up, 5.4 years), Greif et al. found that TAT >200 cm^3 predicted future cardiovascular events independent of risk factors and CAC score (HR 2.1; 95% CI (1.4, 3.2); p=0.01).[125] They also found that adding TAT >200 cm^3 to the model improved the prognostic value above the CAC score alone.[125] In general, cardiovascular fat appears to be an independent risk factor for future CVD and mortality events.

3.2 CORONARY ARTERY AND THORACIC AORTIC CALCIFICATION

3.2.1 Pathophysiology of Calcification

Atherosclerotic calcification is a process that shares features with bone formation and only occurs when other aspects of atherosclerosis are present.[55,126,127] Calcification can occur at any stage of plaque development; however, it is mainly seen in advanced lesions.[55,127] The two primary theories of vascular calcification include a passive model and an active model of formation.[127] The passive model postulates that the inhibitors, present only under homeostatic conditions, prevent calcium formation from occurring.[127] Under this theory, the crystallization of apoptotic cell debris associated with atherosclerotic plaque creates an imbalance in the ionic equilibrium and inhibitors are no longer effective in preventing calcium mineral precipitation.[127] The active model postulates that endothelial and smooth muscles cells in atherosclerotic plaque may originate from bone marrow and are similar to the major cells involved in bone formation.[127] Because of this similarity, these cells have the potential to express proteins and possess signaling pathways to promote osteogenesis.[127] Under normal conditions this expression of proteins is low; however, endothelial cells, smooth muscle cells, inflammatory cytokines, modified lipids, and leukocyte products in atherosclerotic lesions express high levels of these proteins which promote osteogenic differentiation, which in turn may incite an additional inflammatory response.[126-128] Supporting this hypothesis, macrophages have been shown to respond to calcium phosphate crystals which increases inflammation and progresses

atherosclerosis; creating a possible loop between calcification and inflammatory disease progression.[128]

Though the exact biological mechanism between atherosclerosis and arterial calcification is not fully understood, it has been established that arterial calcification is a marker of atherosclerotic disease progression.[55,127,129] Aortic calcification erodes the compliance and elasticity of the artery, increasing cardiac work and promoting congestive heart failure.[126] Coronary artery calcification weakens vasomotor responses and may alter plaque stability.[126,130] Calcification in arteries damages the smooth muscle cells, prevents proper functioning, inhibits homeostasis, and increases the risk of cardiovascular events.[126,129,130]

3.2.2 Quantification of Calcification

All atherosclerotic calcification measurements for this dissertation were measured via EBCT. The EBCT creates tomographic x-ray images with high temporal resolution at 1.5 to 3 mm intervals for CAC and 6 mm for AC over 50 to 100 milliseconds by utilizing a rotating, triggered electron beam.[81,131] Although MDCT is also utilized to measure calcification and has become a more employed mode because of the high spatial resolution and image quality, the EBCT and MDCT methods are highly correlated and both have equivalent reproducibility.[132-134]

A calcified lesion is defined as the presence of at least three connected pixels with an attenuation of greater than 130 HU.[135,136] The traditional method of quantifying CAC burden is via the Agatston score which multiplies the area of the lesion, that meets the criteria listed above, by a weighting factor that is dependent on the highest radiodensity in the lesion (1 for 130-199 HU; 2 for 200-299 HU; 3 for 300-399 HU; and 4 for $\geq$400 HU) and then summed for a total score.[131,135,137] There are other methods of quantifying CAC such as the volume and mass scores that have been utilized in other studies and all three methods are highly correlated with each other.[132,136-139] For the purpose of this dissertation, the Agatston score will be used as the method of assessing atherosclerotic calcification burden because of its established ability to predict future CVD morbidity and mortality and because it is considered to be the standard of reference.[132] Although the Agatston scoring method was originally designed to quantify calcification in coronary arteries, it is the primary means of quantifying calcification in the aorta and the algorithm remains the same.[140]

3.2.3 Epidemiology of the Presence and Progression of Calcification

3.2.3.1 Prevalence and Severity of Calcification

Coronary Artery Calcification

It has been shown that the prevalence and severity of atherosclerotic calcification varies by age, race, and gender with men having more CAC than women and Blacks having less CAC than Whites.[141,142] Determining the distribution of atherosclerotic calcification among these groups is important for understanding the associated risk.[141,142] Among a large multi-ethnic sample of the population in MESA (n=6,110; 53% female; 41% White; 26% Black; mean age, 62 years) 62% of women had zero CAC compared to only 40% in men.[141] On average, White participants were more likely to have CAC compared to Black participants.[141] White women consistently had a higher prevalence of CAC across all age groups when compared to Black women.[141]

Among a large sample of women seen at a preventive medicine facility (n=6,616), CAC was uncommon in women <50 years of age (Agatston CAC score: median (IQR) 0 (0, 0) with only women in the 95th percentile having a CAC score >0.[143] The severity and prevalence of CAC increased with age; however, the median CAC score was < 10 until women were aged >69 years.[143] Consistent with these findings, Janowitz, et al. found that only 10% of women aged <50 years had a CAC score >10.[144] In age stratified analyses, Hoffman, et al. found that among women in the Offspring and Third Generation cohorts of the Framingham Heart Study (n=1,652), the severity of CAC increased with age.[142] Among women aged 45-54 years, 18.8% had an Agatston CAC score >0, 3.1% had a score >100, and 0.5% had a score >400; while

among women aged 55-64 years, 46.5% had a CAC score >0, 11.9% had a score >100, and 4.0% had a score >400; and among women aged 65-74 years, 69.7% had a CAC score >0, 29.9% had a score >100, and 6.5% had a score >400.[142] Among midlife women (mean age, 50.3±2.8 years) in the SWAN Heart Study, 53% of women had an Agatston CAC score =0 and 47% had a CAC score >0.[145]

Thoracic Aortic Calcification

Similarly to CAC, the prevalence and severity of AC increases with age and differs by race and gender.[146,147] In general, men tend to have more AC compared to women and some studies, but not all, have shown that Whites tend to have more AC compared with Blacks.[146,147] Among participants of the Heinz Nixdorf Recall Study (n=4,025; 53% female; mean age, 59±8 years), Kalsch et al. found prevalent AC (Agatston AC score >0) in 63.1% of the population with men having more AC than women (65.2% vs 61.7%, respectively; p=0.009).[147] The mean age among participants with AC was 61.0±8 years compared to 56.6±7 years among those with no AC.[147] Among women, the severity of AC increased with age with a median score of zero through age 54 and then increasing to a median score of 5.3 (IQR: 0.0, 49.6) among women aged 55-59 years; median score of 20.7 (IQR: 0, 104.5) among women aged 60-64 years; and a median score of 53.7 (IQR: 0, 265.9) among women aged 65-69 years.[147] Among women overall (mean age, 59±8 years), 38.8% had an AC score =0; 36.5% had an AC score 1-99; 15.3% had an AC score 100-399; and 9.4% had an AC score $\geq$400.[147]

Consistent with the AC prevalence distribution in the Heinz Nixdorf Recall Study, among women in the SWAN Heart Study (mean age, 50.3±2.8 years) 30% had an Agatston AC score =0 and 70% had an AC score >0.[145] Among participants of MESA (n=6,814; 51% female; mean

age, 63±10 years), Takasu, et al. found that 27% of the population had an Agatston AC score >0 and those with AC presence were older compared to those with no AC (mean age: 71±8 vs 59±9, respectively; p=0.019).[146] In addition, participants with the presence of AC were more likely to be White compared to Black (45% vs 21%, respectively; p<0.001).[146]

3.2.3.2 Calcification Progression

CAC is a strong predictor of future cardiovascular events and with serial measurements over time CAC progression may show the advancement in atherosclerotic burden.[148] Several studies have shown that similar to a single CAC score, CAC progression varies by gender and race.[149,150] Among MESA participants (n=6,810; 53% female; mean age, 62±10 years; mean time between scans; 6.5±3.5 years), Gasset et al. found a mean annualized CAC progression of 23.9 ± 57 Agatston score units (median (IQR) 3.0 (0.3, 21.7)).[149] CAC progression was defined as the absolute change in Agatston CAC score over time. Significant racial and gender differences were found with Black participants having less progression compared to White participants; and women having less progression than men.[149] After adjusting for age, race, sex, and cardiovascular risk factors, Black participants had significantly less CAC progression (-12.2 Agatston units; 95% CI (-15.5 to -8.9); p<0.001) compared to the CAC progression seen among White participants; while men had significantly more CAC progression (+16.4 Agatston units; 95% CI: (+13.9 to +18.9); p<0.001) than seen among women.[149]

Koulaouzidis et al. (n=388; 21% female; mean age, 49±8 years; mean time between scans, 3.0±1.4 years) recently evaluated CAC progression, defined as the absolute change in CAC Agatston scores, among a population with no CAC at baseline.[150] They found a mean CAC progression of 1.6 ± 6.4 (median 0; range 0-80); 75% did not have CAC progression; 20.9% had

an Agatston score increase ranging from 1-10; 3.6% had a score increase ranging from 11-50; and 0.5% had a score increase of >50.[150] The annualized mean CAC progression was 1.8 ± 2.9 (median 0.75; range 0.14-18.0); 24.2% had an annualized progression between 1-10; 0.8% progressed between 1-50; and no participants had an annualized progression >50.[150] The average time from a CAC=0 to a CAC >0 was 4.2 ±1.1 years.[150] Total CAC progression did not differ by gender with men having a mean total CAC progression of 1.8 ±7.1 Agatston units compared to a mean total CAC progression of 0.6 ± 2.0 among women (p=0.11).[150]

3.2.3.3 Calcification and Future Cardiovascular and Mortality Events

CAC Presence and Severity

Coronary artery and aortic calcifications are the most studied measures of subclinical atherosclerosis because they represent the overall burden of atherosclerosis.[148] The associations between CAC and CVD events and mortality have been studied for over 20 years. These studies have shown that both the presence and severity of calcification in the coronary arteries predict future CVD events and all-cause mortality.[151-153] Most recent studies have assessed CAC severity by categorizing CAC scores; however, Jain et al. (n=4,965; 53% female; mean age, 62±10 years) evaluated it as a continuous variable in MESA (**Appendix-Table 1**).[152] They found that for every one standard deviation increase in log(CAC+1) the risk of CHD events (HR 2.4; 95% CI (1.9, 2.8)), heart failure (HR 1.4; 95% CI (1.1, 1.8)), and CVD events (HR 1.7; 95% CI (1.5, 2.0)) significantly increased, after adjusting for traditional CVD risk factors.[152]

In regards to predicting all-cause mortality, two large prospective population-based studies by Blaha et al. (n=44,052; 46% female; mean age, 54±10 years) and by Budoff et al. (n=25,253; 46% female; mean age, 56±11 years) found that participants with CAC scores greater

than 10 at baseline had a greater risk of mortality compared to those with CAC scores of zero, and that all-cause mortality risk increased as CAC score categories increased.[58,151] The findings from these two studies were consistent, except Blaha et al. found mild CAC (Agatston score: 1-10), compared to the absence of CAC, to be significantly associated with all-cause mortality (HR 2.0; 95% CI (1.4, 2.8)), which was not the case in the Budoff et al. study (HR 1.5; 95% CI (0.7, 3.1)).[58,151] The reasons for this inconsistency are unclear since the populations appear similar in many regards; however, the Budoff et al. study reported that 18% of participants with a CAC score 1-10 were on statin therapy.[58] The Blaha et al. study did not report the prevalence of statin therapy and if the study participants differed in this regard, it may be possible that this contributed to the discrepancy.[151]

Since 2005, several studies including the MESA, Early Identification of Subclinical Atherosclerosis by Noninvasive Imaging Research (EISNER), and St. Francis Heart studies have evaluated CAC as a categorical variable and found that the risk of CHD events increased as CAC scores increased.[140,154,155] In large cohorts of midlife men and women with sample sizes ranging from 2,303 to 6,809, compared to CAC scores of either 0 or <10, CAC scores ranging from 100 to 400 were associated with hazard ratios ranging from 9.6 to 11.9 and scores greater than 400 were associated with hazard ratios ranging from 9.9 to 26.2.[140,154,155] When looking at CAC scores ranging from 1-100 in these studies, the results have not been as universally consistent as higher CAC scores have been. Budoff et al. found that CAC scores ranging from 1-100, compared to zero CAC, had a significantly higher risk of CHD (HR 6.1; 95% CI (2.5, 14.7)); however, Arad et al. failed to document significant risk in participants with CAC scores ranging from 1 to 99.[154,155] The inconsistency in results could partly be due to differing covariates and definitions of CHD events. In MESA, Budoff et al. (n=3,923; 61% female; mean age, 58±9

years) conducted analyses by excluding all participants with a CAC score greater than 10 and including only participants who had a CAC score ranging from 0 to 10, to determine if minimal CAC (Agatston scores: 1-10) compared to the absence of CAC predicted CHD events.[153] They found that even people with minimal CAC had an increased risk of incident total CHD events (HR 3.0; 95% CI (1.4, 6.7)) and incident hard CHD events (HR 3.1; 95% CI (1.1, 8.8)) compared to those with no CAC.[153]

Finally, it is important to note that the cut-points for CAC categories, CAC comparison groups, and adjusted variables in multivariable analyses were not the same between studies (**Appendix-Table 1**). Even with the above mentioned variability between studies, CAC has clearly been found to be a significant predictor of CHD events and all-cause mortality. Further, higher levels of CAC have been found to be associated with a greater risk of these events.[56,140,151,155]

Coronary Artery Calcification Progression

Among hypertensive participants, Shemesh et al. (n=210; 46% female; mean age, 64±6 years; mean follow-up, 11.4±4.4 years; mean time between CAC scans, 2.0±0.9 years) evaluated CAC progression and long-term cardiovascular events.[156] They calculated CAC progression as the annualized change in Agatston scores ($CAC_{follow\text{-}up} - CAC_{baseline}$/time between scans) and then categorized participants into three levels of CAC progression: nonprogressors, if annualized CAC change was zero (n=73); slow progressors, if annualized CAC change was below the median (n=78; mean change for slow progressors, 14.4±14); and rapid progressors, if annualized CAC change was the median or above (n=59; mean change for rapid progressors, 154±124).[156] After adjusting for age, sex, baseline total calcium score, hypercholesterolemia, proteinuria, and

creatinine, the slow progressors (HR 1.91; 95% CI (1.1, 3.5)) and rapid progressors (HR 2.1; 95% CI (1.1, 4.0)) had higher risks of cardiovascular events when compared to nonprogressors (p=0.047).[156]

Raggi, et al. (n=495; 37% female; mean age, 57±9 years; mean follow-up, 3.2±0.7 years; mean time between CAC scans, 1.9±1.0 years) evaluated CAC progression and future myocardial infarction, with CAC progression defined as the yearly change in CAC volume score dichotomized into ≥15% versus <15% yearly change among participants taking statin therapy.[157] They found that the risk of myocardial infarction was significantly higher in those with a ≥15% yearly CAC volume change (RR 1.5; 95% CI (4.4, 74.2)) compared to those with <15% change. Interestingly, there was an interaction between baseline CAC volume score and the extent of CAC volume change (p<0.001) such that patients with <15% change had event-free survival of ≥97% at 6 years regardless of baseline CAC volume score; while the relative risks of myocardial infarction among patients with ≥15% CAC volume change were 3.8 (95% CI (1.8, 8.0)) for baseline CAC volume score of 1-400; 6.4 (95% CI (2.7, 14.8)) for baseline CAC volume score of 401-1000; and 12.0 (95% CI (4.5, 32.0)) for baseline CAC volume score ≥1000 (p<0.0001).[157] This suggests the combined importance of both baseline CAC extent and CAC progression, since the risk of myocardial infarction among those with ≥15% annual change increased incrementally with increasing baseline CAC.[157]

Among a large sample of asymptomatic individuals referred for CAC scanning, Budoff, et al. (n=4,609; 27% female; mean age, 60±11 years; mean follow-up, 5.4±3.4 years after the follow-up scan; mean time between CAC scans, 3.1±2.0 years) evaluated the associations between CAC Agatston score progression and all-cause mortality using multiple definitions for CAC progression.[148] For the entire cohort, CAC progression was significantly associated with

all-cause mortality regardless of the method used to calculate CAC progression with hazard ratios ranging from 1.21 to 3.34 (all p<0.0001).[148] The associations between CAC progression and all-cause mortality depended on the baseline CAC score, such that only participants who had a baseline CAC >0 and had CAC progression had a significantly higher risk of death (HR 5.2; 95% CI (3.7, 7.2); p<0.0001) when compared to participants with no baseline CAC and no CAC progression.[148] While participants with baseline CAC, but no CAC progression (HR 1.4; 95% CI (0.99, 2.0); p=0.055) and participants with baseline CAC=0 with CAC progression (HR 0.9; 95% CI (0.4, 2.2)) did not have a significantly higher risk of death compared to participants with no baseline CAC and no CAC progression.[148]

Both of these studies suggest that the risk associated with CAC progression may vary depending on the baseline CAC score, such that if there was severe CAC at baseline and a CAC progression of >15%, then the risk of either a myocardial infarction or death was considerably higher than if there was no CAC or minimal CAC at baseline.[148,157]

Thoracic Aortic Calcification Presence and Severity

Thoracic aortic calcification has not been as thoroughly evaluated as CAC; however several studies have assessed the ability of AC to predict cardiovascular and all-cause mortality events (**Appendix - Table 2**). Much like the statistical analyses involved with CAC, AC and events were defined in several different ways making study comparisons somewhat complex. In regards to the ability of the presence of AC to predict future all-cause mortality events, the results have been inconclusive.[158-161] In a large cohort of predominately white asymptomatic men and women, Santos et al. (n=8,401; 31% female; mean age, 53±10 years) reported AC presence (AC > 0) as a significant predictor of future all-cause mortality when compared to the

absence of AC (HR 1.8; 95% CI (1.2, 2.6)).[160] In another large cohort of men and women, Allison et al. (n=4,544; 43% female; mean age, 57±11 years) found that the presence of AC (AC score >0) compared to the absence of AC was associated with an increased risk of all-cause mortality (HR 2.1; 95% CI (1.2, 3.5)).[159] Lastly, Eisen et al. conducted a smaller study among patients diagnosed with stable angina pectoris (n=361, 15% female; mean age, 62±8 years) and found the risk of all-cause mortality to be more than four-fold higher when AC was present (HR 4.6; 95% CI (1.2, 18.3)).[161] In contrast to these three studies, Kalsch et al. (n=4,040; 53% female; mean age, 59±8 years) failed to document a similar association between AC presence compared to AC absence and future all-cause mortality among participants in the Heinz Nixdorf Recall Study (HR 0.9; 95% CI (0.7, 1.2)).[158]

Much like with all-cause mortality prediction, the associations between the presence of AC and cardiovascular and coronary events were also inconsistent (**Appendix - Table 2**). In gender-stratified analyses in MESA (n=6,807; 53% female; mean age, 62±10 years), Budoff et al. found that AC presence (AC > 0), compared to the absence of AC, was significantly associated with an increased risk of CHD events in women (HR 3.0; 95% CI (1.6, 5.8)) but no statistically significant association was found among men (HR 1.3; 95% CI (0.9, 1.8)).[162] While in the Heinz Nixdorf Study described earlier, Kalsch et al. (n=4,040; 53% female; mean age, 59±8 years) failed to show significant associations between AC presence (AC > 0) and future CHD events (HR 1.4; 95% CI (0.9, 2.1)); however, the definition of events was more stringent compared to the Budoff, et al. study.[158,162]

Most investigators have evaluated AC as a dichotomous variable (presence vs absence); however, two large studies evaluated AC severity and its ability to predict mortality and cardiovascular events.[140,158] In evaluating AC as a continuous measure, Kalsch et al. (n=4,040;

53% female; mean age, 59±8 years) found that the severity of AC was significantly associated with all-cause mortality (HR 1.1; 95% CI (1.0, 1.1); for every one log-unit increase) and myocardial infarction events (HR 1.1; 95% CI (1.0, 1.1); for every one log-unit increase).[158] Wong et al, (n=2,303; 38% female; mean age, 56±10 years) categorized AC score into 4 categories (<10; 10-99; 100-399; >399) and evaluated their associations with CHD and CVD events.[140] Compared to an AC score of less than 10, only AC scores of 100-399 predicted CHD (HR 3.0; 95% CI (1.3, 6.9)) and CVD (HR 2.3; 95% CI (1.0, 5.0)) events.[140]

Overall, results from studies evaluating the presence and severity of AC and future CVD events and all-cause mortality have been inconsistent. Two studies have shown that these associations differ by gender indicating that the associations between AC and all-cause mortality may be stronger in men, while the relationship between AC and CHD events may be stronger in women.[160,162]

3.2.3.4 Traditional Risk Factors for Calcification

Age and Gender

Age and gender are well-established risk factors for both CAC and AC. Numerous studies have found that as age increased the presence, severity, and progression of atherosclerotic calcifications also increased.[58,140,146,163,164] This relationship is consistent in both White and Black men and women, and after adjusting for traditional CVD risk factors.[141,142,165] Though the presence of both CAC and AC increase with age, two separate studies have shown that age is a stronger predictor of AC compared to CAC.[146,164]

Atherosclerotic calcification in the coronary arteries is more prevalent and more severe in men than women, even after adjusting for traditional CVD risk factors.[146,166] The relationship between gender and AC is not as well defined as with CAC.[167] Wong et al. (n=2,647; 36% female; mean age, 53±10 years) found that the unadjusted odds of having AC was lower for women compared to men (OR 0.75; 95% CI (0.58, 0.97)); and Kalsch et al. (n=4,040; 53% female; mean age, 59±8 years) found similar results such that women in the Heinz Nixdorf Recall study had less severe AC (median 13.2 vs 23.7; $p<0.001$; respectively) compared to men.[158,164] In contrast, among two studies looking at unadjusted gender difference, Wong et al. (n=2,303; 38% female; mean age, 56±10 years) found a significant trend that women tended to have higher AC scores compared to men (p for trend=0.02); and Santos et al. (n=8,401; 31% female; mean age, 53±10 years) found similar results such that women were significantly more likely to have AC present than absent (p=0.001).[140,160] Lastly, Takasu et al. (n=6,814; 51% female; mean age, 63±10 years) found that after adjusting for traditional CVD risk factors among

MESA participants, prevalent AC was lower among men than women (Prevalence Ratio: 0.87; 95% CI (0.82, 0.94); p<0.001).[167]

Race as a Risk Factor

Racial differences for the presence and severity of atherosclerotic calcification have been reported for well over a decade. It has been shown that Blacks tend to have a lower prevalence of calcification when compared to Whites; however, not all studies have found this to be true (**Appendix - Table 3**).[146,168] Among MESA participants, (n=6,814; 53% female; mean age, 62±10 years; 38% White, 28% Black), race is a significant predictor of both CAC and AC.[146] Compared to Whites and adjusting for traditional CVD risk factors, Blacks had less CAC (Prevalence Ratio: 0.76; 95% CI (0.72, 0.80)) and less AC (Prevalence Ratio: 0.65; 95% CI (0.59, 0.74)).[146] Additional MESA analyses among only women showed that the associations between race and AC remained significant with Black women having lower relative risks for the presence of AC, compared to White women (RR 0.37; 95% CI (0.29, 0.48)).[167] Other studies found consistent results with Blacks having less CAC than Whites after adjusting for traditional CVD risk factors.[169,170]

It is important to note that not all studies have found a significant racial difference in regards to the presence of CAC. Among young participants in Coronary Artery Risk Development in Young Adults (CARDIA) Study (n=443; 48% female; mean age, 35±4 years; 55% Black, 45% White) and the Dallas Heart Study (n=1,289; 48% female; mean age, 52±6 years; 52% White, 48% Black), there were no statistically significant racial differences for the presence of CAC.[168,171] Among midlife women in SWAN Heart ancillary study (n=540; 100% female; median age, 50±3 years; approximately 39% Black) which includes the population

assessed in this dissertation, the prevalence of CAC and AC were higher among Black women compared to White women; however, these significant differences were only in unadjusted analyses.[172,173]

Race may be a predictor of CAC progression and incident CAC, independent of adiposity, age, gender, and cardiovascular risk factors (**Appendix - Table 3**).[163] Among participants in MESA (n=5,756; 52% female; mean age, 62 years; 40% White; 27% Black; mean time between scans, 2.4 years), Kronmal et al. found that after adjusting for age, sex, BMI, and cardiovascular risk factors, the risk of developing incident CAC was significantly lower for Black participants (RR 0.79; 95% CI (0.65, 0.98); p=0.029) compared to White participants.[163] Among participants with a Agatston CAC score >0 at baseline (n=2,808), the difference in average CAC progression (defined as the absolute difference in CAC scores from baseline to follow-up) for Black participants was significantly lower than White participants (Difference -10.9; 95% CI (-17.1, -4.8); p<0.001) after adjusting for time between scans, age, sex, BMI, and cardiovascular risk factors.[163]

Additional Risk Factors

Several factors have been shown to independently predict the presence of CAC, CAC progression, and the presence of AC, after adjusting for other variables. Some of these include, diabetes, glucose, smoking status, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, total cholesterol, lipid lowering medications, hypertension, systolic blood pressure (SBP) , diastolic blood pressure (DBP), and family history of CVD.[146,167,169,171] These factors tend to hold strong while controlling for age, gender, and race.[146,167,169,171] Risk factors tend to be overall similar between AC and CAC; however, among

MESA participants current smoking and hypertension were stronger independent risk factors for AC than CAC.[146] BMI, a measure of overall adiposity, has been shown to be a modest predictor of CAC, after adjusting for other risk factors; however this association has not been found for AC.[146,167,171,174]

3.2.4 Novel Risk Factors for Calcification

3.2.4.1 Adiponectin, Leptin, C-Reactive Protein

Adiponectin and CAC

Adiponectin has been shown to have anti-inflammatory and anti-atherogenic properties; however, the relationship with CAC is complex and variable.[98,175,176] Maahs, et al. (n=306; 39% female; mean age, 42±8 years; mean time between CAC scans, 2.6 (range 1.6 to 3.3)) evaluated serum adiponectin levels and CAC volume score progression (progression vs no progression).[98] The progression of CAC was dichotomized and considered to be present if the following criterion was met: ($\sqrt{\text{CAC volume}}_{\text{follow-up}} - \sqrt{\text{CAC volume}}_{\text{baseline}}$) $\geq$2.5 units.[98] They found that increased adiponectin levels were associated with the lower odds of having CAC volume score progression (OR 0.32; 95% CI (0.19, 0.56); p<0.001; for every doubling in adiponectin levels) after adjusting for baseline CAC volume score, age, gender, diabetes, SBP, DBP, LDL-C, HDL-C, smoking, BMI, and VAT.[98] These results were consistent for participants with a baseline CAC volume score =0 (OR 0.28; 95% CI (0.12, 0.63); p= 0.002).[98]

In contrast, among nondiabetic participants in the Study of Inherited Risk of Coronary Atherosclerosis (SIRCA) (n=860; 47% female; mean age, 47.9 years), Qasim et al. found no associations between serum adiponectin and Agatston CAC score (logCAC+1) for men or women in gender-stratified analyses when comparing the highest quartile to the lowest quartile of adiponectin (women's ratio 1.36; 95% CI (0.58, 3.19) and men's ratio 1.51 (0.82, 2.76)).[175] To further complicate our understanding of adiponectin and CAC, among CARDIA participants, Steffes et al. (n=2,483; 54% female; 43% Black; 57% White; mean age, 40 years) unexpectedly

found that Agatston CAC score >0 (independent variable) was positively associated with adiponectin (dependent variable) (β (s.e.) 0.085 (0.035); p=0.02) after adjusting for age, race, gender, center, waist circumference, and homeostatic model assessment of insulin resistance (HOMA-IR).[176] Overall, the associations between adiponectin and CAC are not clearly defined or consistent in literature.

Leptin and CAC

Leptin is considered to be primarily pro-inflammatory and pro-atherogenic and is thought to potentially regulate calcification in vascular cells.[97] Similar to adiponectin, the associations between leptin and CAC are not clearly defined.[99,177] Reilly et al. (n=240; 13% female; median age, 61 years) evaluated the associations between plasma leptin levels among participants with type 2 diabetes and found that after adjusting for an extensive set of cardiovascular risk factors, every 5-ng/ml increase in plasma leptin was associated with a higher odds (OR 1.28; 95% CI (107, 1.55); p=0.008) of being in a higher CAC category (0,1-100,101-400,>400) using ordinal logistic regression methods.[99] Among nondiabetic participants in SIRCA (n=860; 47% female; mean age, 47.9 years), Qasim et al. found that serum leptin was associated with CAC (logCAC+1) for men and women in gender-stratified analyses when comparing the highest quartile to the lowest quartile of leptin (women's ratio 3.06; 95% CI (1.28, 7.30); and men's ratio 2.11 (1.13, 3.91)), independent of age, race, family history, exercise, medications, Framingham risk score, and metabolic syndrome.[175] Interestingly, after further adjustment for CRP, the associations between leptin and CAC were attenuated and no longer significant for women (women's ratio 1.99; 95% CI (0.79, 4.99)), but remained significant for men (men's ratio 2.68 (1.40, 5.15)).[175] Further, Iribarren et al. (n=949; 38% female; mean age, 66±3 years) found that

leptin was not associated with the odds of having greater CAC in either women (OR 1.1; 95% CI (0.9, 1.2); p>0.05) or men (OR 0.9; 95% CI (0.7, 1.1); p>0.05) after adjusting for adiposity, cardiovascular risk factors, and CRP.[177] In general, the associations between leptin and CAC remain unclear.

CRP and CAC

Although high CRP levels have been shown to predict future cardiovascular events, the association between CRP and CAC is unclear.[26,96,178] Among participants of the Framingham Offspring Study (n=321; 48% female; mean age, 60±9 years), Wang et al. found that CRP was significantly correlated with CAC Agatston score in men (spearmen correlation 0.22; p<0.01) but not in women (spearmen correlation 0.15; p>0.05) after adjusting for age, SBP, total cholesterol to HDL-C ratio, smoking, diabetes, and BMI.[96] Among postmenopausal women, Redberg et al. (n=172; 100% female; mean age, 64±8 years) found no evidence of an association between CRP and CAC.[178] Similarly, among Dallas Heart Study participants (n=3,373; 44% female; mean age, 45±9 years), Khera et al. found that although CRP was moderately associated with CAC in unadjusted models, all associations were lost after adjusting for cardiovascular risk factors and BMI.[179]

Among Black and White midlife women in the SWAN Heart Study (n=372; 100% female; 36.5% Black; mean age, 51.3 years), CRP was associated with the presence of an Agatston CAC score >0 (OR 1.86; 95% CI (1.52, 2.31); p<0.0001; per 1-log unit increase) and the extent of CAC (β (s.e.) 16.23 (4.16); p<0.0001; per 1-log unit increase) after adjusting for race, site, menopausal status, income, education, and Framingham Risk Score.[180] After further adjustment for BMI the associations were attenuated and no longer significant among White

women.[180] However, CRP was still significantly associated with both the presence of CAC (OR 3.25; 95% CI (1.53, 6.90); p=0.002; per 1-log unit increase) and the extent of CAC (β (s.e.) 19.66 (7.67); p=0.01; per 1-log unit increase) among Black women even after further adjustment for BMI, HOMA-IR, family history of CVD, and medication use.[180] Wang et al. further analyzed the associations between CRP levels and the presence and extent of CAC progression among the SWAN Heart Study population (n=252; 100% female; 32.5% Black; mean age, 51.2±2.6 years; mean follow-up, 2.3 years).[181] The presence of CAC progression was dichotomized and considered to be present if the following criterion was met: 1) women with baseline CAC =0 and follow-up CAC >0; 2) women with baseline 0< CAC <100 and an annualized change of 10 Agatston units at follow-up; or 3) women with baseline CAC ≥100 and an annualized percent change ≥10% (annualized change in CAC score divided by the baseline CAC score); while the extent of CAC progression was calculated using the following equation: [log ($CAC_{(follow\text{-}up)}$ +25) - log($CAC_{(baseline)}$ +25)] / [time (years).[181] They found that CRP was not associated with either the presence of CAC progression (OR 1.1; 95% CI (0.8, 1.5); p=0.6; per 1-log unit increase) or the extent of CAC progression (β (s.e.) 0.003 (0.006); p=0.6; per 1-log unit increase) in the unadjusted analyses.[181]

Although there does not appear to be a strong relationship between CRP and CAC independent of BMI among White women, there is some evidence to suggest that CRP may be relevant among Black women in regards to the presence and extent of CAC.

3.2.4.2 Cardiovascular Fat Quantity

Cardiovascular Fat and CAC Prevalence and Severity

Within the past eight years, research has been focused on determining whether cardiovascular fat depots are risk factors for CVD. Several studies have found significant associations between cardiovascular fat and CAC, after adjusting for cardiovascular risk factors and adiposity measures such as BMI or VAT (**Appendix - Table 4**).[14,82,182] In a cross-sectional study conducted among participants without known CAD (n=215; 39% female; mean age, 58±11 years), each 10 mm^3 increase in EAT volume was significantly associated with a 3.7% increase in CAC score, after adjusting for several traditional CVD risk factors, BMI, and waist circumference.[14] Similarly, in a smaller cross-sectional study (n=111; 32% female; mean age, 60±10 years), Ahmadi et al. found that 10 cm^3 increases in EAT (OR 3.32; 95% CI (1.95, 5.62)), PAT (OR 2.72; 95% CI (1.64, 3.94)), and TAT (OR 3.06, 95% CI (1.87, 5.03)) were individually associated with the increased odds of having a CAC score equal to or greater than 100, after adjusting for several risk factors and BMI.[182]

Among participants of the Offspring cohort of the Framingham Heart Study, Lehman et al. (n=1,067; 56% female; mean age, 59±9 years) found that for every one standard deviation increase in PVAT volume the odds of having the presence of CAC (CAC > 0) was 47% more likely (OR 1.47; 95% CI (1.09, 1.98)), adjusting for risk factors and VAT.[82] Further analyses among these Framingham Heart Study participants by Rosito et al. (n=1,155; 55% female; mean age, 63±9 years) showed that for every one standard deviation increase in EAT volume the odds of having the presence of CAC (CAC > 0) was 21% higher (OR 1.21; 95% CI (1.01, 1.46)), after adjusting for risk factors and VAT; however, no statistically significant associations were found

for TAT (OR 1.23; 95% CI (0.97, 1.57)).[88] Additionally, Huang et al. (n=650; 100% female; mean age, 53±3 years) found that among postmenopausal participants of Kronos Early Estrogen Prevention Study (KEEPS), there was a significant linear trend in the odds of having the presence of CAC across tertiles of TAT and EAT (separate models), after adjusting for BMI and waist circumference (p=0.027, p=0.020; respectively).[10]

Cardiovascular Fat and CAC Progression

Limited research is available evaluating the associations between cardiovascular fat and CAC progression and the results have been inconclusive (**Appendix - Table 4**). In the prospective Heinz Nixdorf Recall study (n=3,367; 53% female; mean age, 59±8 years; mean follow-up, 5.1±0.3 years), Mahabadi, et al. found that after adjusting for age, sex, BMI, and several cardiovascular risk factors, baseline EAT was associated with CAC progression (defined as: $\log(CAC_{follow\text{-}up}+1) - \log(CAC_{baseline}+1)$); with a one standard deviation increase in EAT volume resulting in a 6.1% change in log(CAC+1).[35] Contrary to these findings, among participants of the Rancho Bernando Study (n=598; 76% female; mean age, 68±7 years; mean follow-up, 4.0 years), Wassel et al. did not find associations between EAT or PAT volumes (separate models) and the presence of CAC progression defined as ($\sqrt{CAC\ volume}_{follow\text{-}up} - CAC\ volume_{baseline}$) $\geq$2.5 units in neither minimally adjusted models (age and gender) or fully adjusted models (cardiovascular risk factors, adiposity measures, and adipocytokines).[34] It is interesting to note that although Wassel et al. did not find associations between cardiovascular fat and CAC progression; they did find that PAT, but not EAT, was significantly associated with both the presence and severity of baseline CAC.[34]

Cardiovascular Fat and AC

Limited information is available regarding the associations between cardiovascular fat and AC and the results were not conclusive (**Appendix - Table 4**). The Heart Effects on Atherosclerosis and Risk of Thrombosis in Systemic Lupus Erythematosus (HEARTS) study evaluated the relationship between PVAT and AC among women with and without systemic lupus erythematosus (SLE) using the same PVAT quantification protocol that was used in this dissertation.[83] Shields et al. found that after adjusting for traditional CVD risk factors, inflammatory markers, and regional adiposity, PVAT was associated with the presence of AC (AC >0) for both women with SLE (OR 4.52; 95% CI (1.3, 15.0)) and healthy controls without SLE (OR 4.66; 95% CI (1.8, 12.0)).[83] Consistent with these findings, SWAN Cardiovascular Fat Ancillary Study preliminary analyses among midlife women have shown, irrespective of race or menopausal status, higher volumes of PVAT were associated with greater extent of AC (β (s.e.): 1.60 (0.63), P= 0.01; per 1 log-unit increase in PVAT), after adjusting for several traditional CVD risk factors.[183] In contrast, among participants in the Framingham Heart Study Offspring cohort, Lehman et al. (n=1,067; 56% female; mean age, 59±9 years) found a borderline significant association between PVAT and the presence of AC (AC >0) adjusting for age, sex, and VAT (OR 1.31; 95% CI (1.01, 1.71)); however, with further adjustments, cardiovascular risk factors seemed to explain this association (OR 1.16; 95% CI (0.88, 1.51)).[82]

Overall, cardiovascular fat depots tend to be significant predictors of atherosclerotic calcification after adjusting for traditional risk factors and other adiposity measures, indicating that they may be important predictors of cardiovascular risk. Very limited information is available regarding associations between cardiovascular fat depots and the progression of CAC and nothing in midlife women transitioning through menopause. Understanding how

cardiovascular fat depots influence localized calcification and the progression of calcification in this population may help to elucidate the importance of these fat depots and the cardiovascular risk in midlife women.

3.2.4.3 Cardiovascular Fat Quality

Most recently, research has looked at fat radiodensity as a surrogate marker of adipose tissue quality and a novel risk factor for CVD. To date only four studies have evaluated associations between fat radiodensity and CVD risk and these studies primarily focused on VAT and SAT with limited data on EAT; and the results appear inconsistent.[9,37,45,50] Pracon et al. (n=164; 50% female; mean age, 59±11 years) found that EAT radiodensity was positively associated with CAD (at least one coronary artery stenosis of greater than or equal to 50%) after adjusting for age and X-ray tube voltage, among patients suspected of CAD.[37] In addition, EAT radiodensity was positively associated with CAC score (non-transformed continuous variable), after minimal adjustment for gender, age, smoking status, and X-ray tube voltage.[37]

Three studies among Framingham Heart Study MDCT substudy participants evaluated fat radiodensity and CAC, abdominal aortic calcification, CVD, and all-cause mortality.[9,45,50] Alvey et al. (n=3,079; 49% women; mean age, 50±10 years) evaluated the associations between the radiodensity of several fat depots (VAT, SAT, and EAT) and CAC and abdominal aortic calcification.[45] They found that VAT radiodensity was positively associated with both CAC and abdominal aortic calcification (higher radiodensity values were more adverse); SAT radiodensity was positively associated only with CAC; and EAT was not associated with either measure of subclinical atherosclerosis.[45] Rosenquist et al. (n=3,198; 47% women; mean age, 51±10 years) evaluated associations between VAT and SAT radiodensity and cardiometabolic risk factors.[9]

They found that the lower VAT radiodensity values were associated with a worse cardiovascular risk profile with significant associations with hypertension, impaired fasting glucose, metabolic syndrome, and insulin resistance (higher radiodensity values were more favorable).[5] The results for SAT were less pronounced.[9] An additional study by Rosenquist et al. (n=3,324; 48% women; mean age, 51±10 years) evaluated the associations between fat radiodensity and incident CVD, all-cause mortality, and CVD mortality.[50] They found that VAT and SAT radiodensity values were not associated with incident CVD; but were both positively associated with all-cause mortality and non-CVD mortality (higher radiodensity values more adverse).[50]

In light of the new interest in assessing fat quality, recent studies have also assessed whether the radiodensity of fat is associated with adipokine levels and six-year changes in weight.[184,185] Among a subsample of the Framingham Heart Study participants (n=1,829; 45% female; mean age, 45±6 years), Lee et al. found that independent of several risk factors and the corresponding fat depot volume, both SAT and VAT radiodensity values were inversely associated with leptin and positively associated with adiponectin (higher radiodensity values were more favorable).[185] Among another subsample of the Framingham Heart Study participants (n=835; 40% female; mean age, 46±6 years), Therkelsen, et al. found that after a mean follow-up of 6.1 years, weight gain was inversely associated with VAT and SAT radiodensity (poorer quality of fat).[184] Further, that VAT volume gain was inversely associated with VAT radiodensity (poorer quality of fat), independent of weight change.[184]

No studies have evaluated the associations between TAT and PVAT radiodensity and CVD risk factors and subclinical atherosclerosis in women at midlife. Evaluating novel risk factors for early markers of atherosclerosis, such as cardiovascular fat radiodensity as a surrogate

marker of cardiovascular fat quality among midlife women, may help to elucidate possible mechanisms for the higher rates of CVD seen among postmenopausal women.

3.2.5 Potential Role of Adipokines and Cytokines in Explaining the Associations between Cardiovascular Fat and Calcification

Several studies have shown that EAT expresses less adiponectin and more leptin in people with CAD compared to those without CAD.[93,94,186] Iacobellis, et al. (n=22; 9% female; mean age, 61 years) biopsied EAT from a small sample of patients undergoing surgery (CAD n=16; no CAD n=6) and found that adiponectin protein values (adiponectin/actin ratio) were lower among those with severe CAD compared to those without CAD (1.42 ± 0.77 vs 2.36 ± 0.84; p=0.02; respectively).[93] Among participants undergoing either coronary artery bypass surgery or valve surgery, Eiras, et al. (n=92; 28% female; mean age, 69±8 years) biopsied EAT and found that lower EAT adiponectin expression was positively associated with multi-vessel CAD (OR 0.75; 95% CI (0.61, 0.93); p=0.008) after adjusting for sex, left ventricular ejection fraction, and statin treatment.[94] Cheng et al. (n=58; 21% female; mean age, 61±11 years) biopsied EAT and found that EAT adiponectin expression was significantly lower in patients with CAD compared to those with no CAD (0.41 ± 0.31 μg g^{-1} vs 5.57 ± 0.80 μg g^{-1}; p<0.001; respectively) and EAT leptin expression was significantly higher in CAD patients compared to patients without CAD (90.6 ± 47.1 ng g^{-1} vs 12.3 ± 5.2 ng g^{-1}; p<0.001; respectively).[186]

Although it is often hypothesized that cardiovascular fat depots influence atherosclerotic calcification through secreted adipokines and adipocytokines, limited research is available on whether adiponectin, leptin, and CRP explain the relationships between cardiovascular fat and CAC and AC. Among Rancho Bernando Study participants (n=598; 76% female; mean age, 68±7 years; mean follow-up, 4.0 years), Wassel et al. found that even after adjusting for adipocytokines (interleukin-6, tumor necrosis factor-α, adiponectin, and leptin), PAT was

associated with CAC prevalence and CAC severity.[34] Shields et al. (n=187; 100% female; mean age, 50±10 years) found that PVAT was associated with both CAC and AC in women with and without SLE after adjusting for CRP, fibrinogen, soluble intercellular adhesion molecule, soluble eSelectin, and plasminogen activator inhibitor-1.[83]

Assessing localized adipokine expression and secretion through biopsy is invasive; therefore, identifying a method of assessing this localized effect in a noninvasive manner is important. At this point in time, we are unsure if serum adipokine levels could be used as a surrogate marker of localized cardiovascular fat secretion. Since SAT and VAT depots are considerably larger than cardiovascular fat depots, it would be expected that these depots would contribute more to the systemic levels of adipokines as measured by serum levels. This may have partly contributed to the differences in study findings between the adipokines measured via biopsy compared to adipokines measured in blood serum. More research is needed to assess how serum adipokines influence the associations between cardiovascular fat and atherosclerotic calcifications, especially among midlife women.

4.0 MANUSCRIPT 1: CARDIOVASCULAR FAT IN WOMEN AT MIDLIFE: EFFECTS OF RACE, OVERALL ADIPOSITY, AND CENTRAL ADIPOSITY. THE SWAN CARDIOVASCULAR FAT ANCILLARY STUDY

4.1 ABSTRACT

Background/Objectives: Significant racial/ethnic differences in cardiovascular fat (CF) volumes and in their associations with central adiposity (visceral fat (VAT)) and general adiposity (body mass index (BMI)) have recently been reported among midlife men. Similar research is lacking in midlife women. We assessed whether racial differences in CF volumes and their associations with adiposity measures exist among midlife women. **Subjects/Methods**: A total of 524 women (mean age: 50.9 ± 2.9 years; 62% White and 38% Black) from the SWAN study at the Pittsburgh and Chicago sites were included. BMI, VAT, and CF volumes (epicardial fat (EAT), paracardial fat (PAT), total heart fat (TAT), and aortic perivascular fat (PVAT)) were measured. Analyses were cross-sectional. **Results**: Black women had 19.8% less EAT, 24.5% less PAT, 20.4% less TAT, and 13.2% less PVAT than White women, independent of age, menopausal status, comorbidity, alcohol consumption, and physical activity (P-values <0.001). Differences remained significant after further adjustments for BMI or VAT (P-values <0.05). Race significantly modified associations between adiposity measures and CF volumes. Every 1-SD higher BMI was associated with 66.7% greater PAT in White compared with 42.2% greater PAT in Black women (P-value = 0.004); while, every 1-SD higher VAT was associated with 32.3% greater EAT in Black compared with 25.3% greater EAT in White women (P-value = 0.039). **Conclusions**: Similar to midlife men, racial differences were found in CF volumes and in their associations with adiposity measures among midlife women. Future research should determine how race-specific changes in CF volumes impact cardiovascular risk.

4.2 INTRODUCTION

Cardiovascular fat, defined as fat surrounding the heart and arteries, has been shown to be a metabolically active organ that secretes numerous pro- and anti-inflammatory substances.[62,187] In addition, literature suggests that individual cardiovascular fat depots (epicardial fat (EAT), paracardial fat (PAT), total heart fat (TAT), and thoracic aortic perivascular fat (PVAT)) may be embryonically and metabolically different; therefore, it may be important to look at these depots separately.[21,22] The current theory asserts that cardiovascular fat depots become dysfunctional in states of excess adiposity.[12,187] The close proximity and paracrine effects of cardiovascular fat depots make them potentially important fat depots.[21,62] Indeed, many studies have found cardiovascular fat to be positively associated with the presence and severity of subclinical atherosclerosis, independent of other adiposity measures.[14,34,35,83] Limited information is available regarding determinants of cardiovascular fat, but a few studies have shown positive correlations between cardiovascular fat and adiposity measures (body mass index (BMI) and abdominal visceral fat (VAT)).[15,79,88]

Racial differences in cardiovascular fat volumes have been identified with Black men having less cardiovascular fat compared with White men, independent of overall adiposity.[15] Studies have shown that, in general, Blacks compared with Whites have a more favorable adipose tissue distribution profile with more subcutaneous fat (SAT), and less hepatic fat and VAT, but higher incidence rates of heart failure and stroke, higher rates of diabetes, and a greater risk of cardiovascular disease mortality, indicating a racial-obesity paradox.[15,32,188-193] The recent findings indicating that VAT has a stronger influence on cardiovascular fat in Black compared

with White men introduces a potential mechanism involved in this racial-obesity paradox.[15] It is possible that Black men are better able to buffer excess energy in subcutaneous depots; however, once this storage capacity is exceeded, fat accumulates in ectopic depots at a faster rate in Black compared to White men, potentially increasing their risk of cardiovascular disease.[7,31,86] Interestingly, a recent study among midlife men found that race modified the associations between adiposity measures and individual cardiovascular fat depots, with White men having more EAT and TAT than Black men for increasing BMI levels.[15] Although research regarding racial differences in cardiovascular fat among women is limited, evidence suggests that Black women may have a more favorable fat distribution with lower unadjusted EAT volumes and lower VAT volumes after adjusting for BMI when compared with White women.[31,32,193]

Since postmenopausal women tend to have a higher risk of cardiovascular disease and less favorable fat distribution compared with premenopausal women the relationships between race and adiposity in regards to cardiovascular fat in midlife women are intriguing.[18,52,87] Therefore, our objectives were to determine whether race, overall adiposity, and central adiposity were associated with the quantity of individual cardiovascular fat depots (EAT, PAT, TAT, and PVAT) and evaluate whether cross-sectional associations between individual adiposity measures (BMI, SAT, and VAT) and individual volumes of cardiovascular fat depots vary by race in midlife women. We hypothesized that similar to men, cardiovascular fat volumes would differ by race with Black women having lower volumes of cardiovascular fat compared to White women at midlife; that cardiovascular fat volumes would be positively associated with BMI, SAT, and VAT; and that associations between adiposity measures and cardiovascular fat volumes would differ by race, with stronger associations between BMI and cardiovascular fat found in White women.

4.3 METHODS

Study Population

The Study of Women's Health Across the Nation (SWAN) is a community-based longitudinal multisite study of women transitioning through menopause. The study design and objectives have been reported previously.[194] Briefly, from seven sites (Boston, MA; Detroit, MI; Oakland, CA; Los Angeles, CA; Pittsburgh, PA; Chicago, IL; and Newark, NJ) 3302 participants aged 42-52 years were recruited between 1996 and 1997.[194] The eligibility criteria for the SWAN study included having an intact uterus with at least one ovary, having at least one menstrual period within the past 3 months, and having not been on hormone therapy within the past 3 months. SWAN Heart was an ancillary study conducted to evaluate subclinical atherosclerosis among healthy Black and White women at the Pittsburgh and Chicago study sites.[52] The SWAN Cardiovascular Fat Study was designed to quantify cardiovascular fat among SWAN Heart study participants.[52] A total of 562 out of the 608 SWAN Heart participants who had a readable cardiovascular fat measure (EAT, PAT, TAT, or PVAT) were included in these analyses. Participants were excluded if they were missing adiposity measures or had undergone surgical menopause (n=38). A total of 524 women were included in the PVAT analyses. Due to either poor image quality or scans that did not encompass the designated anatomical boundaries for the EAT, PAT, or TAT depots, 39 additional participants were excluded from EAT, PAT, and TAT analyses, leaving a total of 485 women. All participants signed informed consent and the institutional review board at each site approved the study protocol.

Cardiovascular Fat Depots Measurements and Quantification

EAT, PAT, and TAT volumes were quantified at the Los Angeles Biomedical Research Institute, Harbor-UCLA Medical Center, CA, USA, using images previously acquired during the electron-beam CT scanning to measure coronary artery calcification (3-mm-thick transverse images obtained with a GE-Imatron C150 Electron Beam Tomography scanner (GE Medical Systems, South San Francisco, CA, USA)).[52] EAT, PAT, and TAT volumes were determined from 15 mm above to 30 mm below the superior extent of the left main coronary artery to include the fat around the proximal coronary arteries. The chest wall served as the anterior border and the aorta and the bronchus served as the posterior border. Using volume analysis software (GE Healthcare, Waukesha, WI, USA), adipose tissue was distinguished from the remainder of the heart tissue by a threshold of -190 to -30 Hounsfield units. Cardiovascular fat was measured by manually tracing the borders of the area of interest every 2-3 CT slices beginning at the starting point and then using the software to automatically trace the segments in between these selected slices. As previously described, EAT was defined as the fat inside the pericardium, PAT was defined as the fat outside of the pericardium, and TAT was defined as the total fat within the above described anatomical borders.[52] PAT volume was measured by subtracting the EAT volume from the TAT volume. These fat measures have excellent reproducibility with between- and within-reader spearman correlation coefficients of 0.97.[52]

PVAT was measured using images previously acquired from the electron-beam CT scanning performed to quantify aortic calcification (6-mm-thick cross-sectional images obtained with a GE-Imatron C150 Electron Beam Tomography scanner (GE Medical Systems, South San Francisco, CA, USA)).[52] PVAT was quantified using Slice-o-Matic v4.3 (TomoVision, Magog,

Quebec, Canada) at the University of Pittsburgh Ultrasound Research Lab. PVAT was defined as the adipose tissue surrounding the descending aorta and was distinguished from other tissues by a threshold of -190 to -30 Hounsfield units. The pulmonary bifurcation served as the proximal border, while the first lumbar vertebrae marked the distal border. The vertebral foramen served as the posterior border, while the anterior borders included a horizontal line through the left bronchus which progressed distally until eventually the interior border of the crus of the diaphragm. The borders were manually traced for every slice. This fat measure has excellent intra-reader and inter-reader reproducibility (intra-class coefficient 0.999 and 0.998, respectively).[83]

Adiposity Measures

Abdominal fat was measured with a single 6-mm thick cross-sectional image obtained between the L4 and L5 vertebral space with a GE-Imatron C150 Electron Beam Tomography scanner (GE Medical Systems, South San Francisco, CA, USA) as described elsewhere.[195] Briefly, scans were read by a single reader at the University of Pittsburgh. Adipose tissue was distinguished from other tissues by a threshold of -190 to -30 Hounsfield units using image analysis software (AccuImage software, South San Francisco, CA). A region of interest line along a fascial plane was drawn at the interior of the abdominal musculature and adipose tissue within this area was considered VAT. Subcutaneous fat area was calculated as the difference between the total abdominal fat area and VAT. Excellent inter-observer reliability was reported with intra-class coefficients of 0.94 and 0.97 for VAT and total abdominal fat, respectively.[195] Weight and height were measured in light clothing and without shoes. Weight was measured

using a standardized, calibrated scale and height was measured using a stadiometer. BMI was calculated as weight in kilograms divided by height in square meters.

Study Covariates

Blood pressure was measured in the right arm with the participant seated using a mercury sphygmomanometer after 5 minutes of rest. Blood pressure readings were taken twice and averaged. Hypertension was defined as present if the following criteria were met: SBP $\geq$ 140, or DBP $\geq$ 90, or taking blood pressure medication. Serum glucose was measured using a hexokinase-coupled reaction (Boehringer Mannheim Diagnostics) and diabetes was defined as present if the fasting serum glucose was greater than or equal to 126 or if taking diabetes medication.

Race, age, financial strain, alcohol consumption, cholesterol medication, current smoking status, and physical activity were self-reported. Financial strain was derived from the interview question, "How hard is it for you to pay for the very basics like food, housing, medical care, and heating?" For analyses, the answers were dichotomized as "somewhat hard to very hard" and "not hard at all". Alcohol consumption was categorized into the following: less than or equal to one drink per month; more than one drink per month to one drink per week; and two or more drinks per week. Physical activity was measured via a modified Baecke score of exercise frequency with higher scores indicating more routine physical activity.[196]

Menopausal status was categorized into the following groups using self-reported bleeding patterns: premenopausal (menses in the last 3 months with no change in regularity in the last 12 months); early peri-menopausal (menses in the last 3 months with some change in regularity during the prior 12 months); late peri-menopausal (no menses within the last 3 months, but some

menstrual bleeding over the prior 12 months); and postmenopausal (no menses for the last 12 months). Due to small numbers in some of these categories, premenopausal and early peri-menopausal women were combined in one group and late peri-menopausal and postmenopausal women were combined in another group.[52,197] Women taking hormone therapy were grouped into a separate hormone user group because hormone use could potentially impact bleeding patterns and thus lead to a misclassification, and exogenous hormones in postmenopausal women could inadvertently influence findings.[198]

Statistical Analyses

The characteristics of the study population were summarized and presented as mean ± standard deviation for normally distributed variables; median (interquartile range) for skewed variables; and frequency (percentage) for categorical variables. Normality was assessed for all continuous variables and EAT, PAT, TAT, PVAT, and VAT were log-transformed. Chi-square and t-tests were used to determine whether participant characteristics, cardiovascular fat measures, and adiposity measures differed by race.

Separate univariate linear regression models were created to assess the relationships between the characteristics of the study population and cardiovascular fat volumes. Multivariable linear regression was used to determine whether race as the primary independent variable was associated with cardiovascular fat volumes (EAT, PAT, TAT, and PVAT; separate models). Age, study site, and menopausal status were a priori selected covariates to be included in all analyses. To determine which additional covariates to include in the multivariable analyses, we assessed all variables that were significantly associated with cardiovascular fat volumes using backward elimination. To determine the most parsimonious model, variables

were removed in a stepwise manner based on significance and whether or not they improved the fit of the model. Sensitivity analyses were conducted to determine if triglycerides and low-density lipoprotein cholesterol improved the fit of the model and they did not; therefore, they were not included in the final model. The following covariates were included in the final model: age, study site, menopausal status, diabetes, alcohol consumption, and physical activity. All continuous variables were centered at the mean.

Racial differences in cardiovascular fat volumes were calculated. To provide results that are easily interpreted, % differences and % changes in cardiovascular fat volumes with 95% confidence intervals were calculated.[52,199] Beta coefficients and related 95% confidence intervals from linear regression were presented as the % differences in cardiovascular fat between Blacks and Whites using the formula $(e^{\beta}-1)*100$; and % change in cardiovascular fat per standard deviation in BMI and SAT for Whites and Blacks using the formula $(e^{\beta*SD}-1)*100$.[199] One standard deviation above the geometric mean in VAT was approximately a 55% increase; therefore the following formula was used to calculate the % change in cardiovascular fat per standard deviation in VAT for Whites and Blacks $(e^{\beta*(\log(1.55)}-1)*100$.[199]

Additional adjustments for individual adiposity measures (BMI, VAT, and SAT; separate models) were performed to determine if adiposity measures explained the relationships between race and cardiovascular fat volumes. Scatter plots of the associations between adiposity measures and cardiovascular fat measures by race were created to examine the data. To determine whether race significantly modified the associations between adiposity measures and cardiovascular fat, interactions were assessed between race and adiposity measures as related to cardiovascular fat volumes (separate models) adjusting for the above listed covariates. The race-specific effect sizes of adiposity measures on cardiovascular fat measures (separate models) were

calculated to facilitate comparisons between the effect sizes of adiposity measures on cardiovascular fat in the individual races. Interactions between menopausal status and race in regards to cardiovascular fat volumes were assessed in each model and no statistically significant interactions were found (all $p>0.05$). All analyses were conducted using SAS v9.3 (SAS Institute, Cary, North Carolina).

4.4 RESULTS

The characteristics of the study population overall and by race are presented in **Table 4-1**. The women in our study were 50.9 ± 2.9 years old, 38% Black, and 55% pre/early peri-menopausal. In unadjusted analyses, Black women were more likely to be hypertensive, consume less alcohol, have lower physical activity, have higher BMI levels, have greater SAT, and have lower volumes of EAT and TAT compared with White women. Hypertension, diabetes, BMI, SAT, and VAT were positively associated and physical activity was inversely associated with all four cardiovascular fat volumes (all $p<0.05$), **Supplemental Table 4-1**.

After adjusting for age, study site, menopausal status, hypertension, diabetes, alcohol consumption, and physical activity, Black women had 19.8% less EAT, 24.5% less PAT, 20.4% less TAT, and 13.2% less PVAT than White women (all $p<0.001$) (**Table 4-2**). These racial differences remained significant after further adjustment for BMI and SAT (separate models). Although these significant racial differences persisted after adjusting for VAT, the magnitude of reported effect sizes were somewhat attenuated with Black women having 10.6% less EAT, 11.0% less PAT, 10.2% less TAT, and 5.0% less PVAT than White women (all $p<0.05$).

The scatterplots illustrating the associations between adiposity measures and cardiovascular fat volumes by race are presented in **Supplemental Figure 4-1**. In general, higher levels of adiposity were significantly associated with higher volumes of cardiovascular fat for all depots. Race modified the associations between BMI and PAT, as well as between VAT and EAT. White women had significantly more PAT for higher BMI levels when compared with Black women (interaction p-value=0.004) (**Figure 4-1a**). In contrast, Black women had

significantly more EAT for higher VAT levels when compared with White women (interaction p-value=0.039) (**Figure 4-1b**). The race-specific changes in cardiovascular fat volumes per 1-standard deviation increments in adiposity measures within each race are shown in **Table 4-3**. Every 1 standard deviation higher BMI corresponded to 66.7% greater PAT in White women compared with only 42.2% greater PAT in Black women (interaction p-value=0.004). In contrast, every 1-standard deviation higher VAT corresponded to 32.3% greater EAT in Black women compared with only 25.3% greater EAT in White women (interaction p-value = 0.039). These differences were independent of age, study site, menopausal status, hypertension, diabetes, alcohol consumption, and physical activity.

4.5 DISCUSSION

In a population of White and Black midlife women, we found racial differences in the volumes of cardiovascular fat depots with Black women having significantly less cardiovascular fat in all four depots compared with White women. These racial differences remained significant even after additional adjustment for BMI and SAT (separate models). Although Black women still had significantly lower cardiovascular fat volumes after adjusting for VAT, the results were somewhat attenuated, suggesting a potential role of VAT in understanding racial differences in cardiovascular fat.

In addition, we found that race modified the associations between some of the adiposity measures and cardiovascular fat volumes. The magnitude of the association between BMI and PAT was greater among White women compared with Black women; while the magnitude of association between VAT and EAT was greater among Black women compared with White women. In general, trends towards stronger associations between BMI levels and all four cardiovascular fat volumes were found among White women compared with Black women; while trends towards stronger associations between VAT and cardiovascular fat volumes (except PAT) were found among Black women compared with White women. These findings may support the theory that Blacks are better able to buffer excess energy; however, once the threshold has been met and VAT accumulates, Blacks may be more likely to have higher volumes of cardiovascular fat compared with Whites.[31]

Our findings of Black women having significantly lower cardiovascular fat volumes compared to White women independent of adiposity measures were consistent with results

among men and a population of combined men and women.[15,32] To the best of our knowledge, the ERA-JUMP study conducted among midlife men is the only other study evaluating whether racial differences exist independent of several measures of adiposity, and whether race modifies the associations between adiposity measures and cardiovascular fat volumes. Interestingly, the partial attenuation in the racial differences in cardiovascular fat volumes that we found after adjusting for VAT was similar to the diminution found in the ERA-JUMP population of men.[15] In addition, the interactions reported between race and adiposity measures in our study were comparable to the effect modifications previously reported among men, with the magnitude of associations between BMI and cardiovascular fat volumes greater in Whites compared to Blacks.[15]

Although our results on racial differences in cardiovascular fat volumes were generally consistent with findings previously reported among men, the effect modifications of race on the associations between adiposity measures and cardiovascular fat depots differed in regards to the specific location of the evaluated cardiovascular fat depot.[15] In midlife White men, stronger associations were found between BMI and EAT and BMI and TAT compared to Blacks; however, in midlife White women, higher BMI was significantly associated with greater PAT, but not EAT or TAT, compared with Black women. There are several possible reasons for these discrepancies. Interestingly, we previously showed that greater declines in estradiol over a 4-year period were significantly associated with greater PAT volume, but not EAT or TAT volumes.[52] This suggests that hormones may play an important role in PAT accumulation. Our findings among women differed from the findings among men in another regard. Our study found significantly stronger associations between VAT and EAT among Black women compared to White women; however, only a trend towards this effect modification was found among men.

This discrepancy is most likely due to the small number of Black men and potential lack of power to determine effect modifications for this particular race in the ERA-JUMP study.

Considerable variability exists in the methods, definitions, and assessments of cardiovascular fat depots. Some studies combine the fat inside (EAT) and outside (PAT) the pericardium into one measure and rarely do studies evaluate PAT (using our definition) as a separate fat depot. Literature suggests that the fat inside and the fat outside the pericardium may differ in embryonic origin, adipocyte characteristics, and metabolic activity and, therefore, some researchers suggest assessing each cardiovascular fat depot separately.[21,22] Due to the close proximity, lack of muscle fascia separating it from the myocardium, and the possible shared microcirculation with the coronary arteries, it has been hypothesized that excess EAT may be especially important in regards to cardiovascular risk.[77,200,201] Very little information is available assessing the associations between all three heart depots volumes (EAT, PAT, and TAT; using our definitions) and cardiovascular disease; however, one study among Whites (42% female) found that EAT was more highly correlated with subclinical atherosclerosis than PAT and TAT.[182] Our results support the importance of evaluating cardiovascular fat depots separately.

More research is needed to understand the racial-obesity paradox among Black men and women in regards to cardiovascular disease. Blacks tend to have a more favorable adipose tissue distribution profile with less VAT and cardiovascular fat; however, they tend to have a higher risk of cardiovascular disease and diabetes.[15,32,189,190] In our study population, Black women had 20% less VAT compared to White women after adjusting for BMI ($p<0.001$; data not shown). Despite having less VAT, there was a stronger association between VAT and EAT among Black compared to White women.

Strengths and Limitations

This study has some limitations, including the cross-sectional design which prevented us from assessing temporality. Our population only included Black and White midlife women limiting the generalizability of our results to other races/ethnicities, men, and younger women. In addition, because we did not have percent body fat for this population we used BMI as a surrogate marker of overall adiposity. Our study has several strengths that included the accessibility to data from the well-established parent SWAN study. We had high-quality measurements of cardiovascular fat depots, SAT, and VAT. This is the first study evaluating racial differences in several cardiovascular fat depots independent of separate adiposity measures among women.

4.6 CONCLUSION

In conclusion, Black women had significantly lower volumes of cardiovascular fat compared with White women, independent of individual measures of adiposity. Race modified the associations between adiposity and cardiovascular fat with stronger associations between BMI and PAT in White women compared with Black women, and stronger associations between VAT and EAT in Black women compared with White women. Future studies should determine racial differences in the associations between longitudinal changes in adiposity measures and changes in cardiovascular fat volumes. In addition, research should look at how reductions in individual cardiovascular fat depots influence future CVD risk in different races/ethnicities. Assessing these research questions may help us to better understand the racial-obesity paradox and to identify critical areas for cardiovascular risk reduction.

4.7 TABLES AND FIGURES

Table 4-1: Characteristics of the study population overall and by race

Variables	Total (N=524)	White (n=324) (62%)	Black (n=200) (38%)	P-value
Age, years	50.9 ± 2.9	50.9 ± 2.9	51.0 ± 2.8	0.605
Menopausal Status, n (%)				**0.048***
Pre-/early peri-menopausal	290 (55.3)	183 (56.5)	107 (53.5)	
Late peri-/postmenopausal	183 (34.9)	103 (31.8)	80 (40.0)	
Hormone Users	51 (9.7)	38 (11.7)	13 (6.5)	
Financial Strain, n (%)	158 (31.6)	72 (23.2)	86 (45.5)	**<0.001**
Current Smoker, n (%)	94 (17.9)	56 (17.3)	38 (19.0)	0.619
Alcohol Use, n (%)				**<0.001***
≤ 1/month	196 (37.8)	87 (26.9)	111 (55.5)	
> 1/month to 1/week	191 (36.4)	134 (41.4)	57 (28.5)	
≥ 2/week	135 (25.8)	103 (31.8)	32 (16.0)	
Hypertension, n (%)	133 (25.4)	53 (16.4)	80 (40.0)	**<0.001**
Diabetes, n (%)	26 (5.0)	12 (6.0)	14 (4.3)	0.390
Cholesterol Medication, n (%)	28 (5.3)	16 (4.9)	12 (6.0)	0.600
Physical Activity	7.8 ± 1.7	8.2 ± 1.6	7.2 ± 1.6	**<0.001**
BMI, kg/m^2	29.2 ± 6.2	28.1 ± 5.7	31.0 ± 6.5	**<0.001**
VAT, cm^2	110.6 (72.0, 161.5)	107.6 (69.0, 165.6)	114.0 (79.2, 158.0)	0.956
SAT, cm^2	335.1 ± 151.2	315.6 ± 146.7	362.6 ± 154.3	**<0.001**
EAT, cm^3	36.5 (27.9, 50.5)	38.2 (28.1, 51.6)	35.4 (25.7, 49.7)	**0.014**
PAT, cm^3	9.0 (5.4, 14.8)	8.9 (5.4, 14.8)	9.5 (5.2, 14.8)	0.615
TAT, cm^3	46.7 (34.8, 64.9)	48.0 (35.0, 65.2)	45.2 (33.9, 64.9)	**0.044**
PVAT, cm^3	29.6 (24.1, 39.0)	30.6 (24.5, 39.2)	28.6 (23.0, 37.6)	0.088

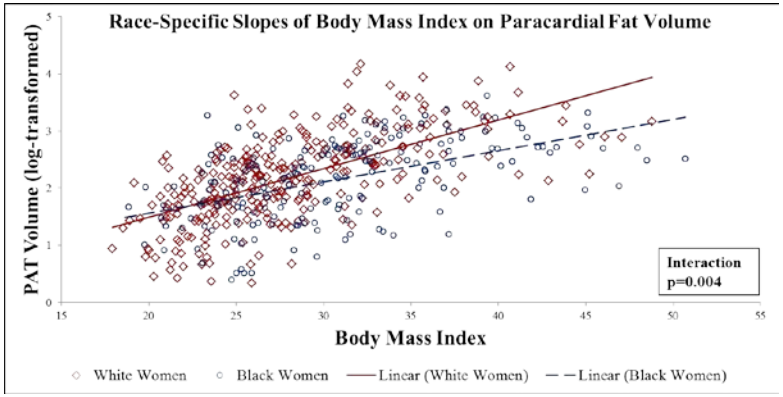
Data presented as mean ± standard deviation, median (interquartile range), or frequency (percentage); *global p-value; BMI, body mass index; VAT, abdominal visceral fat; SAT, abdominal subcutaneous fat; EAT, epicardial fat; PAT, paracardial fat; TAT, total heart fat; PVAT, aortic perivascular fat; Note: cell numbers may not add up to the column total due to missing values for some of the variables.

Table 4-2: Percent differences in volumes of cardiovascular fat depots by race

	EAT (n=485)	**PAT (n=485)**	**TAT (n=485)**	**PVAT (n=524)**
	% Difference (95% CI)	% Difference (95% CI)	% Difference (95% CI)	% Difference (95% CI)
Model 1: adjusted for age, study site, menopausal status, hypertension, diabetes, alcohol consumption, and physical activity				
Black vs White	**-19.8***** (-26.7, -12.4)	**-24.5***** (-34.6, -12.9)	**-20.4***** (-27.3, -12.7)	**-13.2***** (-19.2, -7.0)
Model 2: model 1 + BMI				
Black vs White	**-22.4***** (-27.9, -16.6)	**-28.2***** (-36.3, -19.2)	**-23.2***** (-28.5, -17.6)	**-16.0***** (-20.5, -11.4)
Model 3: model 1 + VAT				
Black vs White	**-10.6**** (-16.6, -4.2)	**-11.0*** (-20.8, -0.1)	**-10.2**** (-16.0, -4.0)	**-5.0*** (-9.7, -0.03)
Model 4: model 1 + SAT				
Black vs White	**-21.0***** (-27.1, -14.5)	**-26.3***** (-35.2, -16.1)	**-21.6***** (-27.8, -15.1)	**-14.0***** (-19.2, -8.6)

*p-value <0.05; **p-value <0.01; ***p-value <0.001; BMI, body mass index; VAT, abdominal visceral fat; SAT, abdominal subcutaneous fat; EAT, epicardial fat; PAT, paracardial fat; TAT, total heart fat; PVAT, aortic perivascular fat; VAT, PVAT, EAT, PAT, and TAT were log transformed; Beta coefficients and related 95% CI from linear regression were presented as % differences between Blacks and Whites using the following formula: $(e^{\beta}-1)*100$.[199]

a.



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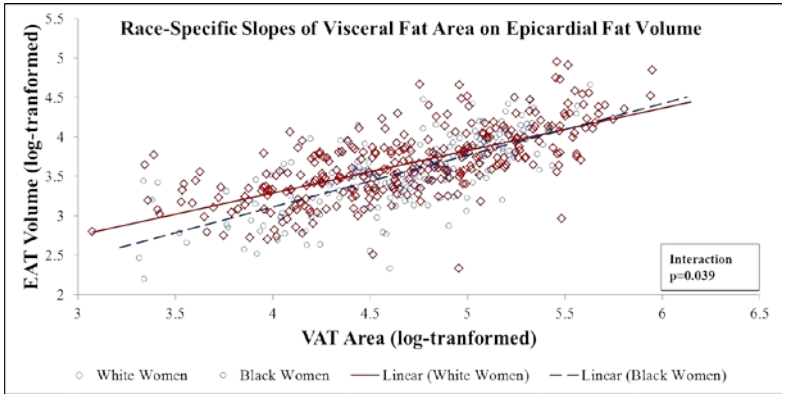

Figure 4-1: Scatterplots of race-specific slopes of BMI on PAT and VAT on EAT

a) BMI on PAT volume by race; b) VAT on EAT volume by race; Interactions between BMI and PAT (p=0.004) and VAT and EAT (p=0.039) were significant.

Table 4-3: Race-specific changes in volumes of cardiovascular fat depots per 1 SD increment changes in adiposity measures

	EAT	PAT	TAT	PVAT
	% Change (95% CI)	% Change (95% CI)	% Change (95% CI)	% Change (95% CI)
Per 1 SD increment of BMI				
Whites	34.9 (28.7, 41.5)	**66.7 (54.2, 79.9)**	40.4 (34.0, 47.1)	31.3 (26.8, 36.0)
Blacks	30.9 (24.5, 37.6)	**42.4 (31.3, 54.5)****	32.5 (26.2, 39.2)	26.0 (21.4, 30.8)
Per 1 SD increment of VAT				
Whites	**25.3 (21.4, 29.2)**	45.7 (38.3, 53.4)	29.2 (25.3, 33.1)	22.3 (19.6, 25.0)
Blacks	**32.3 (26.6, 38.1)***	40.3 (30.5, 50.9)	33.6 (28.1, 39.3)	25.6 (21.7, 29.6)
Per 1 SD increment of SAT				
Whites	21.5 (15.7, 28.0)	43.8 (32.5, 55.8)	25.3 (19.0, 31.7)	17.2 (13.0, 21.5)
Blacks	25.7 (18.8, 32.7)	32.3 (21.0, 44.6)	26.2 (19.4, 33.5)	22.3 (17.0, 27.6)

*Whites differ from Blacks, p-value <0.05; **Whites differ from Blacks, p-value <0.01; BMI, body mass index; VAT, abdominal visceral fat; SAT, abdominal subcutaneous fat; EAT, epicardial fat; PAT, paracardial fat; TAT, total heart fat; PVAT, aortic perivascular fat; EAT, PAT, TAT, PVAT, and VAT were log transformed; all models were adjusted for age, menopausal status, hypertension, diabetes, alcohol consumption, and physical activity; Beta coefficients and related 95% CI from linear regression were presented as % changes in Blacks and Whites using the formula $(e^{\beta*SD}-1)*100$ for BMI and SAT; and the formula $(e^{\beta*(\log(1.55)}-1)*100$ for VAT.[199]

Supplemental Table 4-1: Univariate associations between characteristics of the study population and volumes of cardiovascular fat depots

	EAT		**PAT**		**TAT**		**PVAT**	
	β (s.e.)	p	β (s.e.)	p	β (s.e.)	p	β (s.e.)	p
Black	-0.106 (0.043)	**0.014**	-0.035 (0.070)	0.615	-0.089 (0.044)	**0.044**	-0.057 (0.033)	0.088
Age, years	0.023 (0.007)	**0.001**	0.018 (0.011)	0.128	0.021 (0.007)	**0.004**	0.021 (0.006)	**<0.001**
Menopausal Status		**0.004***		**<0.001***		**0.002***		**0.027***
Pre-/early peri-menopausal	---	---	---	---	---	---	---	---
Late peri-/postmenopausal	0.148 (0.045)	**0.001**	0.204 (0.072)	**0.005**	0.156 (0.046)	**<0.001**	0.093 (0.035)	**0.007**
Hormone Users	0.028 (0.073)	0.696	-0.271 (0.118)	**0.022**	-0.024 (0.075)	0.746	0.046 (0.056)	0.414
Financial Strain	0.028 (0.046)	0.553	0.153 (0.075)	**0.042**	0.053 (0.048)	0.264	0.001 (0.035)	0.975
Current Smoker	-0.048 (0.054)	0.373	0.094 (0.088)	0.284	-0.019 (0.055)	0.734	0.030 (0.042)	0.470
Alcohol Use		0.062*		0.342*		0.059*		0.318*
≤ 1/month	---	---	---	---	---	---	---	---
> 1/month to 1/week	-0.035 (0.049)	0.475	-0.069 (0.080)	0.386	-0.040 (0.050)	0.420	-0.007 (0.037)	0.857
≥ 2/week	-0.123 (0.053)	**0.020**	-0.124 (0.086)	0.149	-0.128 (0.054)	**0.018**	-0.059 (0.041)	0.154
Hypertension	0.196 (0.047)	**<0.001**	0.298 (0.077)	**<0.001**	0.211 (0.048)	**<0.001**	0.141 (0.036)	**<0.001**
Diabetes	0.295 (0.098)	**0.003**	0.490 (0.159)	**0.002**	0.334 (0.100)	**<0.001**	0.301 (0.073)	**<0.001**
Cholesterol Medication	0.120 (0.090)	0.181	0.170 (0.146)	0.244	0.127 (0.092)	0.168	0.101 (0.071)	0.157
Physical Activity	-0.039 (0.012)	**0.002**	-0.087 (0.020)	**<0.001**	-0.048 (0.013)	**<0.001**	-0.024 (0.010)	**0.011**
BMI, kg/m^2	0.044 (0.003)	**<0.001**	0.068 (0.005)	**<0.001**	0.048 (0.003)	**<0.001**	0.038 (0.002)	**<0.001**
VAT, cm^2	0.576 (0.028)	**<0.001**	0.878 (0.048)	**<0.001**	0.634 (0.027)	**<0.001**	0.480 (0.020)	**<0.001**
SAT, cm^2	0.001 (0.0001)	**<0.001**	0.002 (0.0002)	**<0.001**	0.002 (0.0001)	**<0.001**	0.001 (0.0001)	**<0.001**

*global p-value; BMI, body mass index; VAT, abdominal visceral fat; SAT, abdominal subcutaneous fat; EAT, epicardial fat; PAT, paracardial fat; TAT, total heart fat; PVAT, aortic perivascular fat; VAT, PVAT, EAT, PAT and TAT were log transformed.

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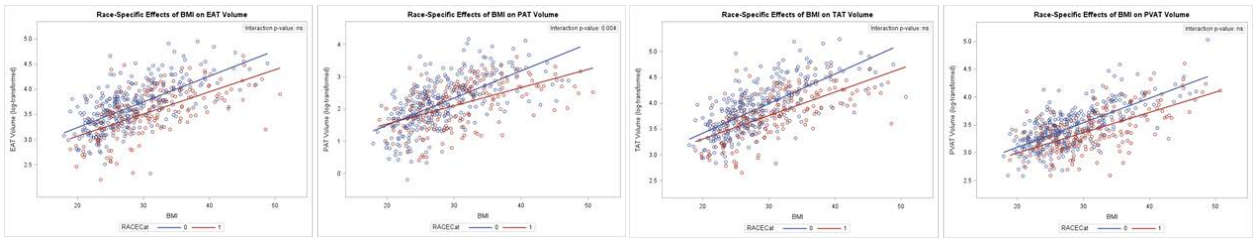

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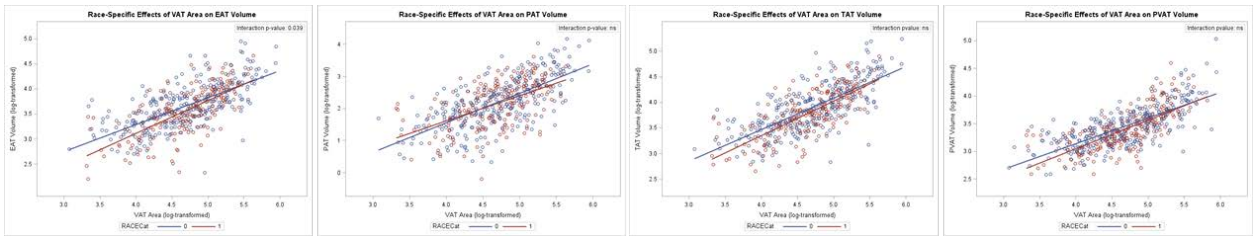

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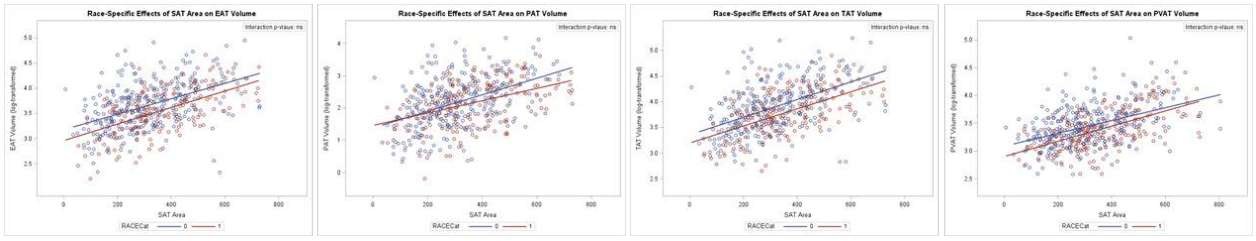

Supplemental Figure 4-1: Scatterplots and regression lines of adiposity measures on cardiovascular fat volumes

a) BMI on cardiovascular fat volumes by race; b) VAT on cardiovascular fat volumes by race; c) SAT on cardiovascular fat volumes by race; 0=White women; 1=Black women; Each adiposity measure was significantly associated with each cardiovascular fat measure (all $p<0.001$). Interactions between BMI and PAT ($p=0.004$) and VAT and EAT ($p=0.039$) were significant.

5.0 MANUSCRIPT 2: CARDIOVASCULAR FAT, ADIPOKINE AND INFLAMMATORY MARKER LEVELS, AND CORONARY ARTERY CALCIFICATION IN MIDLIFE WOMEN: THE SWAN CARDIOVASCULAR FAT ANCILLARY STUDY

5.1 ABSTRACT

Background/Objectives: Cardiovascular fat (CF) is metabolically active and can secrete multiple adipokines which may affect local vasculature. The associations between CF volumes and serum levels of adipokines/inflammatory markers and the potential role of these markers in explaining the associations between CF volumes and subclinical atherosclerosis are not clear in midlife women, a time marked with an increased risk of cardiovascular disease. Our aims were to assess whether CF volumes were associated with serum adipokines/inflammatory markers and whether these associations differed by race. In addition, we evaluated whether these adipokines/inflammatory markers explained associations between CF volumes and coronary artery calcification (CAC) progression.

Subjects/Methods: A total of 313 women (mean age: 51.4 ± 2.7 years; 64% White and 36% Black) from the SWAN study at the Pittsburgh and Chicago sites were included. CF volumes (epicardial fat (EAT), paracardial fat (PAT), total heart fat (TAT)) were measured at baseline. Leptin, adiponectin, leptin to adiponectin (LA) ratio, and c-reactive protein (CRP) were measured a mean 1.0±0.8 year after the baseline visit. CAC was measured at baseline and then after 2.3±0.5 years of follow-up. The annualized extent of CAC progression ([log ($CAC_{(follow-up)}$+25) - log($CAC_{(baseline)}$+25)] / time between scans) was calculated. Linear and logistic regression models were used for statistical analyses. **Results**: EAT, PAT, and TAT (separate models) were positively associated with leptin, LA ratio, and CRP (separate models) after adjusting for cardiovascular risk factors, alcohol consumption, physical activity, and SAT (all $p<0.001$). Adjusting for VAT explained all associations except for the association between PAT

and leptin. Race modified the associations between PAT and leptin and LA ratio, where White women had significantly positive associations, compared to no evident associations among Black women (both $p<0.05$). None of the CF volumes were associated with CAC progression. **Conclusions**: The positive association between PAT and serum leptin, independent of SAT or VAT, suggests that this particular cardiovascular fat depot may contribute to leptin levels in midlife women, independent of other large fat depots.

5.2 INTRODUCTION

Women at midlife experience adipose tissue distribution changes during the menopausal transition that may, in part, contribute to the increased cardiovascular risk seen in postmenopausal women.[17,18,52,53] Adipose tissue is a metabolically active paracrine and endocrine organ that secretes many adipokines with significant influences on several bodily functions.[5,90] The roles of these bioactive substances are complex and often interrelated and have been shown to incite an inflammatory response in states of adipose tissue dysfunction.[63,91] During periods of caloric surplus, adipose tissue is designed to buffer the excess energy through adipocyte enlargement and recruitment.[65] When the fat depot is no longer able to recruit new adipocytes, the current adipocytes continue to expand and become hypertrophic.[65,92] These hypertrophic adipocytes reduce the amount of oxygen and blood supply to the fat depot causing cell death and macrophage recruitment.[4,65] In addition, the size of hypertrophic adipocytes have been shown to be directly associated with the increased secretion of pro-inflammatory adipokines and the reduction of anti-inflammatory adipokines production.[92]

Because of the close proximity of cardiovascular fat to the heart and vasculature, it has been hypothesized that a crosstalk between cardiovascular fat and the heart and vasculature may occur with adipocyte secreted proteins and hormones directly migrating into the bordering cells or being released through the vasa vasorum.[22,95] Adiponectin and Leptin are two of the most prolific and commonly researched adipokines that are secreted directly from adipocytes.[63] Adiponectin is generally considered to be anti-inflammatory and has been shown to be inversely associated with insulin resistance and atherosclerosis.[23,24,63,101] Leptin is considered to be pro-

inflammatory with some evidence suggesting that leptin may be associated with cardiometabolic risk; however, the findings have not been consistent.[99,202-205] Interestingly, the ratio between leptin to adiponectin (LA ratio) has been suggested as a potentially better marker of cardiometabolic risk than the individual adipokine levels.[81,206] Other adipocytokines, such as interleukin-6, have been shown to stimulate an inflammatory response resulting in increased c-reactive protein (CRP) levels.[91] In some studies, CRP has been associated with cardiovascular events and mortality, and may help to improve the cardiovascular risk classification when added to traditional risk factors.[26-28] Interestingly, the levels of adipokines and cytokines have been shown to vary by race, with Blacks generally having a less favorable profile with lower adiponectin levels and higher leptin and CRP when compared with Whites.[29,30]

Previous work from the SWAN Cardiovascular Fat Study showed that late peri-/postmenopausal women had higher epicardial fat (EAT), paracardial fat (PAT), and total heart fat (TAT) volumes than pre-/early peri-menopausal women, independent of several risk factors, physical activity, and obesity.[52] Recently, studies have focused on evaluating the localized effects of cardiovascular fat depots on the heart and vasculature, independent of other adiposity measures, such as abdominal visceral fat (VAT).[14,88] In fact, some studies have found that cardiovascular fat depots are independently associated with atherosclerotic disease burden measured via coronary artery calcification (CAC), cardiovascular events, and all-cause mortality.[14,121,182] Only a few studies have evaluated whether cardiovascular fat is associated with CAC progression and the results have been inconsistent.[34-36] It is unclear whether adipokines and cytokines explain the associations between cardiovascular fat and subclinical atherosclerosis and the progression of CAC.[34]

Due to the less favorable fat distribution and increased cardiovascular risk in midlife women, it is important to understand these associations in this vulnerable population.[17,18,52] Therefore, our objectives were to determine whether cardiovascular fat depot volumes (EAT, PAT, and TAT) were associated with serum adiponectin, leptin, CRP, and the LA ratio, independent of other abdominal adiposity measures (visceral fat (VAT) and subcutaneous fat (SAT)); to determine whether the proposed associations between the individual cardiovascular fat depot volumes and the individual adipokine and inflammatory marker levels varied by race in midlife women; to assess whether individual cardiovascular fat depot volumes (separate models) were associated with the presence and extent of CAC progression (separate models); and to determine whether individual adipokine and inflammatory marker levels explained the proposed associations between cardiovascular fat depots and CAC progression.

5.3 METHODS

Study Population

The Study of Women's Health Across the Nation (SWAN) is a longitudinal study of women transitioning through menopause. The study design and objectives have been reported previously.[194] Briefly, 3302 participants between the ages of 42 to 52 years old from seven cities (Pittsburgh, PA; Boston, MA; Los Angeles, CA; Oakland, CA; Chicago, IL; Detroit, MI; and Newark, NJ) were recruited from 1996 to 1997.[54] In order to be eligible for the SWAN study, women needed to meet the following criteria: have an intact uterus, have at least one ovary, have at least one menstrual period with the past 3 months, and have not been on hormone therapy within the past 3 months. The SWAN Heart Ancillary study (n=608) was designed to assess subclinical atherosclerosis among healthy White and Black SWAN participants at the Pittsburgh and Chicago study sites and includes a baseline and a 2.3 ± 0.5 year follow-up visit.[52] The SWAN Cardiovascular Fat Study was conducted to evaluate cardiovascular fat among SWAN Heart study participants who had CT scans (n=562) at the baseline visit.[52] Participants were excluded from these analyses if they did not have a readable cardiovascular fat measure (EAT, PAT, or TAT) (n=41); if they were missing all four adipokine and inflammatory marker measures (leptin, adiponectin, LA ratio, and CRP) (n=197); and if they had undergone surgical menopause (n=11). A total of 313 women were included in the cardiovascular fat and adipokine analyses. Due to missing either baseline CAC or follow-up CAC measure, an additional 91 participants were excluded from the CAC progression analyses, leaving a total of 222 women.

Sensitivity analyses were completed to determine if the women excluded from analyses differed from the women included in analyses. For the adipokine analyses, the 249 women excluded were younger, were less likely to be taking cholesterol medication, and had higher volumes of PAT, compared with the 313 women included in analyses (all $p<0.05$). For the CAC progression analyses, the 340 women excluded were younger, were less likely to be taking cholesterol medication, had higher PAT volumes, were more likely to have diabetes, consumed more alcohol, and were less likely to have the presence of CAC progression (all $p<0.05$), compared with the 222 women included in analyses. The study protocol was approved by institutional review board at each site and all participants signed informed consent.

Cardiovascular Fat Depots Quantification

The Los Angeles Biomedical Research Institute, Harbor-UCLA Medical Center, CA, USA, quantified EAT, PAT, and TAT volumes using images previously attained during the electron-beam CT scanning to measure CAC from 3-mm-thick transverse images using a GE-Imatron C150 Electron Beam Tomography scanner (GE Medical Systems, South San Francisco, CA, USA).[52] In order to measure the fat around the proximal coronary arteries, volumes of EAT, PAT, and TAT were measured from 15 mm above to 30 mm below the superior extent of the left main coronary artery. The anterior border was the chest wall and the posterior border was the aorta and the bronchus. Using volume analysis software (GE Healthcare, Waukesha, WI, USA), adipose tissue was distinguished from other tissues using a threshold of -190 to -30 Hounsfield units. As previously described, EAT was defined as the fat inside the pericardium, PAT was defined as the fat outside of the pericardium, and TAT was defined as the total fat within the above listed anatomical borders.[52] PAT volume was quantify by subtracting the EAT volume

from the TAT volume. The between- and within-reader spearman correlation coefficients were at least 0.97 for these cardiovascular fat measures.[52]

Adiposity Measures

A single 6-mm thick cross-sectional image from a GE-Imatron C150 Electron Beam Tomography scanner (GE Medical Systems, South San Francisco, CA, USA) was used to measure abdominal fat between the L4 and L5 vertebral space as previously described.[195] Briefly, adipose tissue was distinguished from other tissues by a threshold of -190 to -30 Hounsfield units using image analysis software (AccuImage software, South San Francisco, CA) at the University of Pittsburgh. VAT was defined as adipose tissue interior to the abdominal musculature. Subcutaneous fat area (SAT) was calculated by subtracting VAT from the total abdominal fat area. Excellent inter-observer reliability was reported with intra-class coefficients of 0.94 for VAT and 0.97 for total abdominal fat.[195] Weight was measured using a standardized scale and height was measured using a stadiometer. BMI was calculated as weight in kilograms divided by height in square meters.

CAC Quantification

Calcification of the coronary arteries was measured using an Imatron C-150 Ultrafast electron-beam CT scanner (GE-Imatron, South San Francisco, CA) at each site. Measurements were taken from the level of the aortic root to the apex of the heart resulting in 30 to 40 contiguous 3-mm-thick transverse images. Images were taken using electrocardiographic triggering to obtain 100-ms exposures during the same phase of the cardiac cycle during maximal breath holding (60% of the RR interval). The Agatston method was utilized to score

images using a DICOM workstation and AccuImage, Inc. software (South San Francisco, CA) at the University of Pittsburgh.[135] Calcification was considered present if there were at least 3 contiguous pixels with a radiodensity ≥130 HU. CAC was quantified individually for the 4 major coronary arteries and then summed for a total Agatston score. This protocol has excellent reproducibility with an intra-class correlation coefficient of 0.99.[207] CAC was measured at the SWAN Heart baseline study (the baseline visit for the SWAN Cardiovascular Fat Ancillary Study) and again after 2.3 ± 0.5 years of follow-up.

Blood Assays

Cardiovascular disease risk factors were assayed at the Medical Research Laboratories (Lexington, KY, USA), as previously described.[208] Briefly, triglyceride levels were analyzed using enzymatic methods on a Hitachi 747 analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN, USA). High-density lipoprotein cholesterol (HDL-C) was isolated using heparin-2M manganese chloride and low-density lipoprotein cholesterol (LDL-C) was calculated according to the Friedewald equation.[209] Serum insulin was measured using a radioimmunoassay (RIA; DPC Coat-a-count, Los Angeles, CA, USA) procedure and glucose was measured using a hexokinase-coupled reaction (Boehringer Mannheim Diagnostics). Diabetes was defined as a fasting serum glucose greater than or equal to 126 or taking diabetes medication. The homeostatic model assessment of insulin resistance index (HOMA-IR) was calculated from insulin and glucose as (fasting insulin (mU/L) x fasting glucose (mmoles/L))/22.5.[210] Using an ultra-sensitive rate immunonephelometric method, CRP levels were measured (BN 100, Dade-Behring, Marburg, Germany).[29] Serum leptin and adiponectin levels were measured in duplicate using colorimetric enzyme immunoassay kits (Millipore, St.

Charles, MO) at the University of Michigan. The lower limit of detection and mean coefficient of variation percent for duplicate samples for each subject, respectively, were: adiponectin: 0.78 ng/mL, 4%; and leptin: 0.5 ng/mL, 4%). Serum adipokine levels were only available for one time point in the SWAN parent study. Serum adipokine levels were measured a mean 1.2 ± 0.6 years after the cardiovascular fat measurements.

Study Covariates

Age, race, highest educational level achieved, household income, financial strain, alcohol consumption, medication usage, current smoking status, and physical activity were self-reported at the SWAN Heart baseline visit. Financial strain was derived from the interview question, "How hard is it for you to pay for the very basics like food, housing, medical care, and heating?" For analyses, the answers were dichotomized as "somewhat hard to very hard" and "not hard at all" due to small numbers in certain categories. Alcohol consumption was categorized into the following: less than or equal to one drink per month; more than one drink per month to one drink per week; and two or more drinks per week. Total annual household income level was categorized into following groups: less than $50,000; at least $50,000 but less than $75,000; and at least $75,000. Highest educational level achieved was grouped into the following categories: high school or less; some college/vocational school; and college degree or greater. Physical activity was measured via a modified Baecke score of exercise frequency in which higher scores represented more frequent routine physical activity.[196] Blood pressure readings using a mercury sphygmomanometer after 5 minutes of rest were taken using the right arm with the participant seated. Two blood pressure readings were measured and then averaged. Hypertension was

defined as present if the following criteria were met: SBP $\geq$ 140, or DBP $\geq$ 90, or taking blood pressure medication.

Menopausal status was based on self-reported bleeding patterns and included the following categories: premenopausal (menses within the last 3 months, but no change in regularity in the last 12 months); early peri-menopausal (menses in the last 3 months with some change in regularity during the prior 12 months); late peri-menopausal (no menses within the last 3 months, but some menstrual bleeding over the prior 12 months); and postmenopausal (no menses for the last 12 months). To account for small numbers in some of these groups, premenopausal and early peri-menopausal women were combined into one category and late peri-menopausal and postmenopausal women were combined into another category.[52,197] Lastly, women taking hormone therapy were grouped into a separate group termed hormone users because hormone use has the potential to impact bleeding patterns and could inadvertently affect findings.[198]

Statistical Analyses

Cardiovascular Fat Volumes and Adipokine and Inflammatory Marker Levels

The characteristics of the study population were summarized and presented as mean ± standard deviation for normally distributed variables; median (interquartile range) for skewed variables; and frequency (percentage) for categorical variables. Normality was assessed for all continuous variables and EAT, PAT, TAT, VAT, LDL-C, HOMA-IR, triglycerides, leptin, adiponectin, LA ratio, and CRP were all log-transformed. Chi-square and t-tests were used to determine whether participant characteristics, cardiovascular fat volumes, and adiposity measures differed by race.

Separate univariate linear regression models were created to assess the relationships between the characteristics of the study population and cardiovascular fat volumes, as well as between the characteristics of the study population and the adipokine and inflammatory marker levels. Multivariable linear regression was used to determine whether individual cardiovascular fat volumes (EAT, PAT, and TAT; separate models) as the primary independent variables were associated with individual adipokine and inflammatory marker levels (leptin, adiponectin, LA ratio, and CRP; separate models) as the primary dependent variables. Age, race, study site, menopausal status, and time interval (time between dates of the cardiovascular fat scans and dates of the adipokine/inflammatory marker measurements) were a priori selected covariates to be included in all analyses. To determine which additional covariates were to be included in the multivariable analyses we used backward elimination procedures and assessed all variables significantly associated with both the independent variable (any of the cardiovascular fat volumes) and the dependent variable (any of the adipokine and inflammatory marker levels) at a $p<0.20$ significance level. In order to determine the most parsimonious model, variables were removed in a stepwise manner based on significance and whether or not they improved the fit of the model. The following covariates were selected for inclusion in the multivariable models: alcohol consumption, physical activity, systolic blood pressure (SBP), HDL-C, and log-transformed HOMA-IR. Further adjustments for adiposity measures (VAT and SAT; separate models) were conducted to determine associations between cardiovascular fat volumes and adipokine and inflammatory marker levels, independent of other adiposity measures.

Interactions between race and cardiovascular fat volumes (separate models) as related to adipokine and inflammatory marker levels were assessed. For ease of interpretation, interactions

were presented as percent differences with 95% confidence intervals between White and Black women for every 20% increment change in cardiovascular fat volumes.

Cardiovascular Fat Volumes and CAC Progression

The annualized extent of CAC Progression was calculated using the following equation: [log($CAC_{(follow\text{-}up)}$+25) - log($CAC_{(baseline)}$+25)] / [time (years) between baseline and follow-up scans], similar to previous studies.[163] CAC progression was considered present if any of the following criteria were met: 1) women with baseline CAC =0 and follow-up CAC >0; 2) women with baseline 0< CAC <100 and an annualized change of 10 Agatston units at follow-up; or 3) women with baseline CAC >100 and an annualized percent change ≥10% (annualized change in CAC score divided by the baseline CAC score).[211] Chi-square and t-tests were used to determine whether participant characteristics, cardiovascular fat volumes, and adipokine and inflammatory maker levels differed by the presence of CAC progression.

Separate univariate logistic and linear regression models were created to assess the relationships between the characteristics of the study population and both the presence of CAC progression and the annualized extent of CAC progression, respectively. Multivariable logistic and linear regression were used to determine whether individual cardiovascular fat volumes (EAT, PAT, and TAT; separate models) as the primary independent variables were associated with the presence of CAC progression and the annualized extent of CAC progression as the primary dependent variables, respectively. The same model building procedures as listed above were conducted, resulting in the following covariates included in the regression models: age, study site, menopausal status, time interval (time between baseline and follow-up scans), log-transformed (baseline CAC+1), log-transformed triglycerides, and hypertension. Further

adjustments for individual adipokine and inflammatory marker levels (leptin, adiponectin, LA ratio, and CRP; separate models) were conducted to determine whether the potential associations between cardiovascular fat volumes and CAC progression (presence of CAC progression and annualized extent of CAC progression) could be explained by adipokine and inflammatory marker levels. Due to multicollinearity, adjusting for VAT made the models unstable and, therefore, we were unable to include VAT as a covariate in those analyses. Interactions between race and cardiovascular fat volumes (separate models) in regards to the presence and annualized extent of CAC progression (separate models) were assessed and no statistically significant interactions were found (all $p>0.05$). All analyses were conducted using SAS v9.3 (SAS institute, Cary, North Carolina).

5.4 RESULTS

Characteristics of the Study Population

The characteristics of the study population overall and by race are presented in **Table 5-1**. The women in our study were 51.4 ± 2.7 years old, 36% Black, and 55% pre-/early peri-menopausal. In unadjusted analyses, Black women were more likely to be hypertensive with higher SBP and diastolic blood pressure (DBP) levels, to consume less alcohol, to take blood pressure or cholesterol medication, to have lower physical activity, to have lower household income, to have lower triglyceride levels, to have higher BMI and HOMA-IR levels, and to have greater SAT compared with White women (all $p<0.01$). In addition, when compared with White women, Black women had a less favorable adipokine and inflammatory maker profile with higher leptin, LA ratio, and CRP, and lower adiponectin levels (all $p<0.01$).

Cardiovascular Fat Volumes and Adipokine and Inflammatory Marker Levels

Menopausal status, SBP, DBP, hypertension, LDL-C, triglycerides, HOMA-IR, diabetes, BMI, SAT, and VAT were positively associated, and physical activity and HDL-C were inversely associated with all three cardiovascular fat volumes (all $p<0.05$) (**Supplemental Table 5-1)**. Race, SBP, DBP, hypertension, triglycerides, HOMA-IR, BMI, VAT, SAT, EAT, PAT, and TAT were all associated with *less* favorable levels of all four adipokine and inflammatory markers (all $p<0.05$) (**Supplemental Table 5-2**). More frequent alcohol consumption, higher levels of physical activity, and higher HDL-C levels were all associated with *more* favorable levels of all four adipokine and inflammatory markers (all $p<0.05$).

After adjusting for age, study site, menopausal status, time interval, physical activity, alcohol consumption, SBP, HDL-C, and log-transformed HOMA-IR, all three cardiovascular fat volumes were positively associated with higher levels of leptin, LA ratio, and CRP (separate models; all $p<0.001$) (**Table 5-2**). After adjusting for SAT, the results were somewhat attenuated; however EAT, PAT and TAT remained positively associated with higher levels of leptin, LA ratio, and CRP (separate models; all $p<0.05$). When models were adjusted for VAT instead of SAT, all associations were attenuated except the association between PAT and leptin. None of the cardiovascular fat volumes were associated with adiponectin levels. Interactions between race and cardiovascular fat volumes were assessed and the percent differences between Blacks and Whites for each 20% increment change in cardiovascular fat are presented in **Table 5-3**. Race modified the associations between PAT and leptin and LA ratio, such that White women had significantly positive associations, compared with no evident associations among Black women (both interaction p-values <0.05). These differences were independent of age, menopausal status, time interval, SBP, physical activity, alcohol consumption, HDL-C, log-transformed HOMA-IR, and VAT. Additional significant interactions between race and EAT and TAT in relation to leptin and LA ratio were found (all $p<0.05$) with White women having more leptin and LA ratio for higher amounts of EAT and TAT, when compared with Blacks; however, EAT and TAT were not associated with leptin and LA ratio in either Whites or Blacks. There were no significant effect modifications by race found for the associations between cardiovascular fat volumes and adiponectin or CRP levels.

Cardiovascular Fat Volumes and CAC Progression

The mean time between the baseline scan and the follow-up CAC scan was 2.3 ± 0.5 years. The characteristics of the study population by the presence of CAC progression for participants with CAC progression measures and adipokine and inflammatory marker levels are presented in **Supplemental Table 5-3**. Approximately 21.2% of women (n=47) in our study had CAC that progressed from the baseline to follow-up scans out of the 222 women included in these subgroup analyses. In unadjusted analyses, women who had CAC progression had higher SBP, DBP, triglyceride, BMI, and LA ratio levels and lower adiponectin levels; were more likely to be hypertensive and to take blood pressure and cholesterol medication; to have higher baseline Agatston CAC scores; and to have greater VAT, when compared to women with no CAC progression (all $p<0.05$). These findings were consistent with the univariate associations found between characteristics of the study population and the annualized extent of CAC progression (**Supplemental Table 5-4**). The odds for the presence of CAC progression and the beta coefficients and standard errors for the annualized extent of CAC progression derived from multivariable logistic and linear regression, respectively, are shown in **Table 5-4**. No associations were found between cardiovascular fat volumes and the presence of CAC progression and the annualized extent of CAC progression

5.5 DISCUSSION

Among White and Black midlife women, we found that cardiovascular fat volumes (EAT, PAT, and TAT) were significantly associated with serum leptin, LA ratio, and CRP levels, independent of cardiovascular risk factors, physical activity, and alcohol consumption. These associations were somewhat attenuated after further adjustment for SAT, however they remained statistically significant. However, after adjusting for VAT, only PAT and serum leptin were significantly associated. This suggests that PAT may play a role in systemic serum leptin levels. Most interestingly, we found that race modified the associations of PAT with leptin and LA ratio, such that there were significant associations among Whites and no evident associations among Blacks, independent of cardiovascular risk factors and VAT. In addition we found no significant associations between cardiovascular fat volumes and CAC progression in a subsample of midlife women. Due to the lack of associations between cardiovascular fat and adiponectin, we suspect that the associations found among cardiovascular fat and LA ratio were driven primarily by the associations with leptin.

Limited research is available on the associations between cardiovascular fat volumes and serum leptin, adiponectin, LA ratio, and CRP. Our findings that cardiovascular fat volumes were associated with CRP were consistent with the Framingham Heart Study, where EAT and PAT (using our terminology) were positively associated with serum CRP independent of clinical covariates and BMI and waist circumference; and with the Rancho Bernardo Study where they found no associations between cardiovascular fat depots and serum adiponectin.[212] In our unadjusted analyses we found that all three cardiovascular fat depots were inversely associated

with adiponectin and positively associated with leptin, which differed from the findings in the Rancho Bernardo Study where they found no unadjusted associations between cardiovascular fat volumes and serum adipokines.[34] Although the exact reason for our inconsistent findings is unclear, it is possible that differences in the study populations may have contributed. Our population only included Black and White women, was considerably younger and healthier with less smoking rates, hypertension, diabetes prevalence, and subclinical atherosclerosis compared to the Rancho Bernardo study participants.[34]

Recent evidence has shown that the cardiovascular fat depots secrete numerous pro- and anti-inflammatory substances making them active endocrine and paracrine organs.[60-62] The potential mechanisms of how cardiovascular fat depots could contribute to serum levels of adipokines and inflammatory markers are unclear; however, these depots may become dysfunctional in states of excess adiposity.[4,61] In a physiological state, fat cells enlarge in response to excess energy.[65] This response signals the proliferation of new adipocytes from precursor cells.[65] When adipocyte hyperplasia is impaired, existing adipocytes continue to buffer fatty acids resulting in extreme hypertrophy and dysfunctional adipose tissue.[4,65] As these hypertrophic adipocytes continue to enlarge, the increased distance from the vasculature and reduced capillary density can prevent the adipocytes from getting enough blood flow and oxygen resulting in hypo-perfusion and hypoxia.[65-67] This hypoxic state causes an inflammatory response with macrophage recruitment.[41,67,68] In addition, hypertrophic adipocytes have been shown to shift towards secreting predominately pro-inflammatory adipokines and cytokines and may incite increased levels of inflammatory markers such as CRP.[4,21]

Our findings that race modified the associations between PAT and leptin are intriguing. To the best of our knowledge, no other studies to date have evaluated the effect modification of

race for these associations. We anticipated finding stronger associations between cardiovascular fat volumes and adipokine and inflammatory marker levels among Black women compared to White women, which would have helped to elucidate the racial-obesity paradox seen with cardiovascular disease. Since Black women tend to have a more favorable fat distribution profile, but a less favorable adipokine and inflammatory marker profile, finding a greater magnitude of association between cardiovascular fat volumes and adipokine and inflammatory marker levels in Black women could potential help us to understand some of the reasons why Blacks have higher cardiovascular disease; however, this was not the case in our study.[29,32,52,188,190] Contrary to our hypotheses, we found stronger associations between PAT and leptin and LA ratio (separate models) among White women compared with Black women, independent of cardiovascular risk factors, alcohol consumption, physical activity, and VAT. In our previous work we found that White women had more cardiovascular fat and VAT than Black women after adjusting for other adiposity measures.[213] It is possible that the higher volumes of PAT and VAT among White women contributed more to the serum leptin levels compared with smaller cardiovascular fat depots. The higher levels of adipokines seen among Black women in our study may have been driven by the greater amounts of SAT for this population.[214] More research is necessary to investigate the potential mechanisms behind the racial-obesity cardiovascular disease paradox.

In this subsample of midlife women, we found no associations between individual cardiovascular fat volumes and the presence or annualized extent of CAC progression. Although several other studies have found associations between cardiovascular fat volumes and the prevalence and severity CAC, the findings regarding the associations between cardiovascular fat depots and CAC progression have been inconsistent.[14,34-36,182] In a population of middle age

White men and women, EAT (using our fat depot definition) was positively associated with CAC progression; and in a multi-ethnic population of midlife men and women, EAT (using our fat depot definition) was associated with the odds of having CAC progression.[35,36] However, in a multi-ethnic predominately older female population (n=598; 76% female; mean age, 68±7 years), neither EAT nor PAT (using our fat depot definitions) were associated with the odds of having CAC progression.[34]

The lack of association in our study is most likely due to the lack of CAC progression in this subsample of midlife women which could be because of the limited follow-up time (2.3 ± 0.5 years). A recent study evaluating the prevalence of CAC progression among an asymptomatic population with no CAC, suggests a follow-up period of at least 4 years in order to adequately capture CAC progression.[150] In addition, the SWAN Cardiovascular Fat Ancillary study population was healthy with minimal CAC at baseline (median score 0.00; IQR (0.00, 6.18)) and at follow-up (median score 0.00; IQR (0.00, 12.30)). Lastly, the sample size of this population was considerably reduced for the CAC progression analyses since it was limited to participants with adipokine and inflammatory marker levels as well. Due to the lack of associations between cardiovascular fat volumes and CAC progression in this study, we were unable to assess the importance of adipokines and inflammatory marker levels in understanding these associations. Some studies have shown that even minimal CAC (Agatston scores 1-10) is associated with incident CHD; therefore, even the minimal CAC progression found among this population may be an important risk factor.[153] More research is necessary with a longer follow-up time, other measures of subclinical atherosclerosis, and among a population with greater CAC to understand the influence of cardiovascular fat on CAC progression.

Strengths and Limitations

This study has some limitations, including the cross-sectional design which prevented us from assessing temporality. Even though we had a follow-up CAC score, cardiovascular fat volumes and adipokine and inflammatory markers were only measured at one time point. The median time between cardiovascular fat volume measurements and adipokine and inflammatory marker measurements was 1.0 ± 0.8 years; ideally we would have had these measures completed at the same time point and at follow-up. Our population only included Black and White midlife women limiting the generalizability of our results to men, younger women, and other races/ethnicities. In addition, we had a short follow-up time between baseline and follow-up CAC scans and minimal CAC progression in our population. Our study has several strengths that included available data from the well-respected SWAN parent study. We had several adipokine and inflammatory marker levels available and high-quality measurements of cardiovascular fat depots, SAT, and VAT. This is the first study evaluating how race modifies the associations between cardiovascular fat volumes and adipokine and inflammatory marker levels.

5.6 CONCLUSION

In conclusion, cardiovascular fat volumes were positively associated with serum leptin, LA ratio, and CRP, independent of age, race, physical activity, cardiovascular risk factors and SAT. However, adjusting for VAT attenuated all associations with the exception of those between PAT and leptin. In addition, race modified the associations of PAT with leptin and LA ratio such that significant associations were found among Whites, but not among Blacks, independent of cardiovascular risk factors and VAT. This further illustrates that the PAT depot may be relevant among midlife women. Future studies should evaluate this cardiovascular fat depot separately and should evaluate how PAT accumulation affects cardiovascular risk.

5.7 TABLES AND FIGURES

Table 5- 1: Characteristics of the study population overall and by race

Variables	N	Total (N=313)	White (n=201/64.2%)	Black (n=112/(35.8%)	P-value
Age, years	313	51.4 ± 2.7	51.4 ± 2.8	51.4 ± 2.7	0.997
Pittsburgh Site, n (%)	313	156 (49.8)	83 (41.3)	73 (65.2)	**<0.001**
Menopausal Status, n (%)	310				0.108*
Pre-/early peri-menopausal		171 (55.2)	111 (55.8)	60 (54.0)	
Late peri-/postmenopausal		100 (32.3)	58 (29.2)	42 (37.8)	
Hormone Users		39 (12.6)	30 (15.1)	9 (8.1)	
Education, n (%)	310				0.648*
≤ High school		51 (16.4)	34 (17.1)	17 (15.3)	
Some college/vocational school		151 (48.7)	93 (46.7)	58 (52.2)	
≥ College degree		108 (34.8)	72 (36.2)	36 (32.4)	
Household Income, n (%)	311				**<0.001***
< $50k		97 (31.2)	50 (25.0)	47 (42.3)	
≥ $50k to < $75k		78 (25.1)	44 (22.0)	34 (30.6)	
≥ $75k		136 (43.7)	106 (53.0)	30 (27.0)	
Financial Strain, n (%)	310	96 (31.0)	45 (22.6)	51 (46.0)	**<0.001**
Current Smoker, n (%)	310	46 (14.8)	27 (13.6)	19 (17.1)	0.399
Alcohol Consumption	310				**<0.001***
≤ 1/month		129 (41.6)	65 (32.7)	64 (57.7)	
> 1/month to 1/week		111 (35.8)	80 (40.2)	31 (27.9)	
≥ 2/week		70 (22.6)	54 (27.1)	16 (14.4)	
Physical Activity	310	7.80 ± 1.64	8.05 ± 1.65	7.37 ± 1.55	**<0.001**
SBP, mmHg	304	118.7 ± 16.0	114.5 ± 13.8	126.0 ± 17.1	**<0.001**
DBP, mmHg	304	75.8 ± 10.0	73.1 ± 8.7	80.4 ± 10.6	**<0.001**
Hypertension, n (%)	303	78 (25.7)	32 (16.7)	46 (41.4)	**<0.001**
HOMA-IR	273	2.02 (1.47, 3.14)	1.81 (1.40, 2.74)	2.70 (1.70, 4.76)	**<0.001**
Diabetes, n (%)	310	13 (4.2)	9 (4.5)	4 (3.6)	0.699
HDL-C, mg/dL	295	57.2 ± 14.2	57.3 ± 14.2	57.1 ± 14.1	0.884
LDL-C, mg/dL	283	116.0 (96.0, 139.0)	114.0 (98.0, 141.0)	117.0 (93.5, 132.0)	0.410
Triglycerides, mg/dL	286	102.5 (77.0, 140.0)	108.5 (84.0, 156.0)	92.5 (70.0, 118.0)	**<0.001**

Table 5-1: Continued

Cholesterol Medication, n (%)	310	22 (7.1)	12 (6.0)	10 (9.0)	0.327
BP/Cholesterol Medication, n (%)	313	69 (22.0)	34 (16.9)	35 (31.2)	**0.003**
BP Medication, n (%)	310	57 (18.4)	26 (13.1)	31 (27.9)	**0.001**
BMI, kg/m^2	302	29.3 ± 6.2	28.1 ± 5.6	31.3 ± 6.9	**<0.001**
VAT, cm^2	307	113.4 (72.4, 164.7)	113.0 (72.0, 166.9)	115.23 (77.4, 158.2)	0.829
SAT, cm^2	307	341.1 ± 153.2	322.4 ± 150.6	374.6 ± 152.8	**0.004**
EAT, cm^3	313	37.0 (27.9, 52.6)	38.4 (28.2, 52.9)	36.0 (25.9, 52.5)	0.130
PAT, cm^3	313	8.42 (4.63, 14.51)	8.42 (4.63, 14.36)	8.60 (4.63, 14.73)	0.973
TAT, cm^3	313	46.8 (34.3, 66.5)	47.4 (34.9, 65.9)	45.2 (33.3, 66.8)	0.213
Leptin, ng/mL	300	29.0 (17.0, 44.9)	24.8 (14.2, 39.4)	38.6 (21.5, 51.3)	**<0.001**
Adiponectin, µg/mL	302	11.95 (9.16, 16.47)	13.82 (10.62, 18.90)	9.53 (7.06, 12.35)	**<0.001**
LA Ratio	300	2.46 (1.23, 4.40)	1.81 (0.96, 3.54)	3.75 (2.27, 6.65)	**<0.001**
CRP, mg/dL	201	1.95 (0.90, 4.60)	1.80 (0.80, 4.00)	2.60 (1.30, 6.40	**0.002**
Time Interval, years	301	1.02 ± 0.77	1.04 ± 0.72	0.99 ± 0.85	0.607

*Global p-value; SBP, systolic blood pressure; DBP, diastolic blood pressure; BP/Cholesterol medication, taking blood pressure or cholesterol lowering medication; hypertension, SBP ≥140 or DBP ≥90 or taking blood pressure medication; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; diabetes, maximum glucose ≥126 or taking diabetes medication; HOMA-IR, homeostatic model assessment of insulin resistance; BMI, body mass index; VAT, abdominal visceral fat; SAT, abdominal subcutaneous fat; EAT, epicardial adipose tissue; PAT, paracardial adipose tissue; TAT, total heart adipose tissue; LA ratio, leptin to adiponectin ratio; CRP, high-sensitivity c-reactive protein; time interval, time in years between cardiovascular fat scan and adipokine/inflammatory marker measurements; EAT, PAT, TAT, VAT, LDL-C, triglycerides, HOMA-IR, leptin, adiponectin, LA ratio, and CRP were log-transformed to approach normality; note: cell numbers may not add up to the column total due to missing values for some of the variables.

Table 5-2: Associations between cardiovascular fat volumes and adipokine/inflammatory marker levels using multivariable linear regression

	Leptin (n=300)		Adiponectin (n=302)		LA Ratio (n=300)		CRP (n=201)	
	β (s.e.)	p	β (s.e.)	p	β (s.e.)	p	β (s.e.)	p
EAT								
Model 1	0.693 (0.080)	<**0.001**	-0.271 (0.053)	<**0.001**	0.952 (0.104)	<**0.001**	0.861 (0.137)	<**0.001**
Model 2	0.499 (0.094)	<**0.001**	-0.059 (0.061)	0.330	0.486 (0.114)	<**0.001**	0.520 (0.172)	**0.003**
Model 3	0.217 (0.085)	**0.012**	-0.055 (0.064)	0.387	0.271 (0.109)	**0.013**	0.324 (0.176)	0.067
Model 4	0.023 (0.097)	0.812	-0.0061 (0.071)	0.931	0.030 (0.120)	0.803	0.281 (0.201)	0.163
PAT								
Model 1	0.501 (0.048)	<**0.001**	-0.149 (0.033)	<**0.001**	0.643 (0.064)	<**0.001**	0.565 (0.085)	<**0.001**
Model 2	0.367 (0.054)	<**0.001**	0.005 (0.037)	0.889	0.360 (0.067)	<**0.001**	0.352 (0.103)	<**0.001**
Model 3	0.232 (0.050)	<**0.001**	0.015 (0.039)	0.699	0.216 (0.065)	**0.001**	0.221 (0.107)	**0.041**
Model 4	0.144 (0.060)	**0.017**	0.062 (0.044)	0.154	0.081 (0.074)	0.274	0.214 (0.124)	0.086
TAT								
Model 1	0.750 (0.076)	<**0.001**	-0.267 (0.051)	<**0.001**	1.006 (0.096)	<**0.001**	0.912 (0.131)	<**0.001**
Model 2	0.513 (0.090)	<**0.001**	-0.039 (0.059)	0.515	0.549 (0.110)	<**0.001**	0.579 (0.167)	<**0.001**
Model 3	0.288 (0.084)	<**0.001**	-0.032 (0.063)	0.614	0.318 (0.107)	**0.003**	0.370 (0.174)	**0.035**
Model 4	0.087 (0.100)	0.387	-0.034 (0.073)	0.640	0.053 (0.124)	0.667	0.352 (0.207)	0.090

Model 1: age, race, site, menopausal status, and time interval (time in years between cardiovascular fat scan and adipokine /inflammatory marker measurements); Model 2: model 1 + physical activity, alcohol, systolic blood pressure, high-density lipoprotein cholesterol, and homeostatic model assessment of insulin resistance; Model 3: model 2 + abdominal subcutaneous fat; Model 4: model 2 + abdominal visceral fat; epicardial fat, paracardial fat, total heart fat, abdominal visceral fat, homeostatic model assessment of insulin resistance, leptin, adiponectin, leptin to adiponectin ratio, and c-reactive protein were log transformed.

Table 5-3: Percent differences for interactions between cardiovascular fat and race as related to adipokine/inflammatory marker levels for each 20% increment change in cardiovascular fat volumes

	Leptin		Adiponectin		LA Ratio		CRP	
	% Change (95% CI)	p-value	% Change (95% CI)	p-value	% Change (95% CI)	p-value	% Change (95% CI)	p-value
EAT								
EAT	-3.3 (-7.9, 1.5)	0.169	1.4 (-2.1, 5.0)	0.444	-4.6 (-10.1, 1.2)	0.120	4.6 (-5.5, 15.6)	0.384
EAT*Whites	6.4 (0.7, 12.3)	**0.027**[*]	-2.4 (-6.2, 1.6)	0.235[*]	8.9 (1.8, 16.4)	**0.013**[*]	1.1 (-9.7, 13.2)	0.851[*]
PAT								
PAT	0.012 (-2.9, 3.0)	0.994	1.8 (-0.3, 4.0)	0.105	-1.7 (-5.3, 1.9)	0.342	2.8 (-3.3, 9.4)	0.373
PAT*Whites	4.3 (0.9, 7.8)	**0.012**[*]	-1.1 (-3.4, 1.4)	0.390[*]	5.4 (1.2, 9.8)	**0.012**[*]	1.8 (-5.1, 9.1)	0.617[*]
TAT								
TAT	-2.4 (-7.0, 2.5)	0.334	2.2 (-1.4, 5.8)	0.239	-4.4 (-9.9, 1.5)	0.141	5.8 (-4.4, 17.0)	0.274
TAT*Whites	6.6 (1.1, 12.4)	**0.018**[*]	-2.4 (-6.1, 1.4)	0.218[*]	9.2 (2.3, 16.5)	**0.008**[*]	1.3 (-9.3, 13.1)	0.817[*]

[*]Interaction p-value for the effect modification in the associations between race and cardiovascular fat volumes on adipokine/inflammatory marker levels; log-transformed β coefficients and related 95% CI from linear regression were presented as % differences between Black and White women for a 20% increment change in respective cardiovascular fat volume in relation to adipokine/inflammatory marker levels using the following formula: $(e^{\beta*\log(1.2)}-1)*100$;[199] EAT, epicardial fat; PAT, paracardial fat; TAT, total heart fat; LA ratio, leptin to adiponectin ratio; CRP, c-reactive protein; all models were adjusted for age, site, menopausal status, time interval (time in years between cardiovascular fat scan and adipokine /inflammatory marker measurements), physical activity, alcohol consumption, systolic blood pressure, high-density lipoprotein cholesterol, homeostatic model assessment of insulin resistance, and abdominal visceral fat; EAT, PAT, TAT, abdominal visceral fat, homeostatic model assessment of insulin resistance, leptin, adiponectin, LA ratio, and CRP were log transformed

Table 5-4: Multivariable associations between cardiovascular fat and measures of CAC progression independent of adipokines

	Extent of CAC Progression		Odds of CAC Progression	
Variables	β (s.e.)	p	OR (95% CI)	p
EAT				
Model 1	0.021 (0.015)	0.177	1.39 (0.72, 2.71)	0.332
Model 2	0.014 (0.016)	0.364	1.24 (0.63, 2.46)	0.535
Model 3	-0.015 (0.019)	0.436	0.66 (0.29, 1.51)	0.323
Model 3 + Leptin	-0.017 (0.021)	0.416	0.74 (0.30, 1.82)	0.510
Model 3 + Adiponectin	-0.015 (0.019)	0.430	0.68 (0.29, 1.59)	0.370
Model 3 + LA Ratio	-0.020 (0.021)	0.335	0.63 (0.26, 1.56)	0.320
Model 3 + CRP	-0.015 (0.020)	0.445	0.75 (0.31, 1.80)	0.519
PAT				
Model 1	0.015 (0.009)	0.107	1.20 (0.80, 1.80)	0.391
Model 2	0.012 (0.010)	0.230	1.08 (0.70, 1.66)	0.723
Model 3	-0.0035 (0.011)	0.761	0.75 (0.45, 1.24)	0.256
Model 3 + Leptin	-0.0035 (0.013)	0.765	0.78 (0.44, 1.41)	0.416
Model 3 + Adiponectin	-0.0024 (0.012)	0.843	0.76 (0.45, 1.29)	0.305
Model 3 + LA Ratio	-0.0055 (0.013)	0.672	0.69 (0.39, 1.24)	0.218
Model 3 + CRP	-0.0032 (0.012)	0.797	0.81 (0.47, 1.40)	0.448
TAT				
Model 1	0.022 (0.015)	0.142	1.37 (0.72, 2.60)	0.331
Model 2	0.015 (0.015)	0.314	1.21 (0.63, 2.33)	0.574
Model 3	-0.014 (0.018)	0.457	0.64 (0.28, 1.42)	0.266
Model 3 + Leptin	-0.016 (0.021)	0.439	0.70 (0.29, 1.73)	0.444
Model 3 + Adiponectin	-0.013 (0.019)	0.477	0.66 (0.29, 1.51)	0.326
Model 3 + LA Ratio	-0.019 (0.020)	0.350	0.60 (0.25, 1.45)	0.256
Model 3 + CRP	-0.014 (0.019)	0.467	0.72 (0.31, 1.70)	0.454

Model 1: unadjusted; Model 2: adjusted for age, race, site, menopausal status, and time interval (time in years between baseline scans and follow-up scans); Model 3: model 2 + hypertension, log-transformed triglycerides and log-transformed ($CAC_{baseline}+1$); EAT, epicardial fat; PAT, paracardial fat; TAT, total heart fat; CRP, c-reactive protein; LA ratio, leptin to adiponectin ratio; extent of CAC progression was defined using the following equation:$[\log(CAC_{(follow\text{-}up)}+25) - \log(CAC_{(baseline)}+25)]$/time (years) between baseline and follow-up scans; EAT, PAT, TAT, triglycerides, leptin, adiponectin, LA ratio, CRP, and baseline CAC+1 were log-transformed to approach normality

Supplemental Table 5-1: Univariate associations between characteristics of the study population and cardiovascular fat volumes

	EAT		**PAT**		**TAT**	
Variables	β (s.e.)	p	β (s.e.)	p	β (s.e.)	p
Age, years	0.022 (0.010)	**0.020**	0.035 (0.016)	**0.032**	0.023 (0.010)	**0.022**
Black	-0.084 (0.055)	0.130	-0.003 (0.093)	0.973	-0.072 (0.058)	0.213
Chicago site	-0.042 (0.053)	0.432	0.320 (0.087)	**<0.001**	0.023 (0.055)	0.677
Menopausal Status		**0.012**[*]		**<0.001**[*]		**0.003**[*]
Pre-/early peri-menopausal	---	**---**	---	**---**	---	**---**
Late peri-/postmenopausal	0.161 (0.058)	**0.006**	0.284 (0.096)	**0.003**	0.178 (0.060)	**0.003**
Hormone users	-0.036 (0.082)	0.665	-0.333 (0.135)	**0.014**	-0.088 (0.085)	0.306
Education		0.997[*]		0.477[*]		0.918[*]
≤ High school	---		---		---	---
Some college/vocational school	0.005 (0.75)	0.950	0.133 (0.125)	0.288	0.030 (0.078)	0.700
≥ College degree	0.001 (0.079)	0.988	0.153 (0.131)	0.244	0.031 (0.818)	0.708
Household Income		0.142[*]		0.607[*]		0.196[*]
< $50k	---	---	---	---	---	---
≥ $50k to < $75k	0.080 (0.071)	0.262	0.106 (0.119)	0.374	0.082 (0.074)	0.267
≥ $75k	-0.052 (0.062)	0.401	0.006 (0.104)	0.954	-0.043 (0.065)	0.505
Financial Strain	0.045 (0.058)	0.440	0.200 (0.096)	**0.038**	0.073 (0.060)	0.228
Current Smoker, n (%)	-0.056 (0.075)	0.460	0.066 (0.126)	0.603	-0.037 (0.079)	0.634
Alcohol Consumption		**0.008**[*]		**0.055**[*]		**0.008**[*]
≤ 1/month	---	---	---	---	---	---
> 1/month to 1/week	-0.070 (0.060)	0.246	-0.175 (0.101)	0.082	-0.089 (0.062)	0.155
≥ 2/week	-0.214 (0.069)	**0.002**	-0.258 (0.116)	**0.026**	-0.222 (0.071)	**0.002**
Physical Activity	-0.068 (0.016)	**<0.001**	-0.133 (0.026)	**<0.001**	-0.079 (0.016)	**<0.001**
SBP, mmHg	0.006 (0.002)	**0.002**	0.012 (0.003)	**<0.001**	0.007 (0.002)	**<0.001**
DBP, mmHg	0.008 (0.003)	**0.005**	0.016 (0.004)	**<0.001**	0.009 (0.003)	**0.002**
Hypertension	0.169 (0.061)	**0.006**	0.302 (0.102)	**0.003**	0.192 (0.064)	**0.003**
HDL-C, mg/dL	-0.009 (0.002)	**<0.001**	-0.016 (0.003)	**<0.001**	-0.010 (0.002)	**<0.001**

Supplemental Table 5-1: Continued

LDL-C, mg/dL	0.309 (0.102)	**0.003**	0.404 (0.174)	**0.021**	0.327 (0.106)	**0.002**
Triglycerides, mg/dL	0.325 (0.054)	**<0.001**	0.492 (0.093)	**<0.001**	0.349 (0.056)	**<0.001**
Cholesterol Medication	0.062 (0.104)	0.554	0.057 (0.174)	0.743	0.051 (0.109)	0.641
BP/Cholesterol Medication	0.107 (0.064)	0.096	0.190 (0.107)	0.076	0.120 (0.066)	0.072
BP Medication	0.138 (0.069)	**0.047**	0.216 (0.115)	0.061	0.152 (0.072)	**0.034**
HOMA-IR	0.318 (0.041)	**<0.001**	0.496 (0.073)	**<0.001**	0.344 (0.043)	**<0.001**
Diabetes	0.410 (0.132)	**0.002**	0.616 (0.221)	**0.006**	0.436 (0.137)	**0.002**
BMI, kg/m^2	0.044 (0.004)	**<0.001**	0.072 (0.006)	**<0.001**	0.048 (0.004)	**<0.001**
VAT, cm^2	0.566 (0.036)	**<0.001**	0.968 (0.059)	**<0.001**	0.641 (0.035)	**<0.001**
SAT, cm^2	0.0014 (0.00016)	**<0.001**	0.0023 (0.00026)	**<0.001**	0.0015 (0.00016)	**<0.001**

Data presented as linear regression beta coefficients (standard error); *global p-value; SBP, systolic blood pressure; DBP, diastolic blood pressure; BP/Cholesterol medication, taking blood pressure or cholesterol lowering medication; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; diabetes, defined as maximum glucose $\geq$ 126 or taking diabetes medication; hypertension, defined as SBP $\geq$ 140 or DBP $\geq$ 90 or taking blood pressure medication; HOMA-IR, homeostatic model assessment of insulin resistance; BMI, body mass index; VAT, abdominal visceral fat; SAT, abdominal subcutaneous fat; EAT, epicardial adipose tissue; PAT, paracardial adipose tissue; TAT, total heart adipose tissue; EAT, PAT, TAT, VAT, LDL-C, triglycerides, and HOMA-IR were log-transformed

Supplemental Table 5-2: Univariate associations between characteristics of the study population and adipokine/inflammatory marker levels

Variables	Leptin (n=300)		Adiponectin (n=302)		LA Ratio (n=300)		CRP (n=201)	
	β (s.e.)	p	β (s.e.)	p	β (s.e.)	p	β (s.e.)	p
Age, years	0.025 (0.016)	0.108	0.0065 (0.010)	0.512	0.017 (0.021)	0.414	0.034 (0.024)	0.160
Black	0.410 (0.086)	**<0.001**	-0.394 (0.052)	**<0.001**	0.797 (0.112)	**<0.001**	0.413 (0.135)	**0.002**
Chicago site	0.339 (0.084)	**<0.001**	-0.110 (0.054)	**0.044**	0.438 (0.115)	**<0.001**	-0.002 (0.132)	0.988
Menopausal Status		**0.016**[*]		0.278[*]		**0.016**[*]		0.482[*]
Pre-/early peri-menopausal	---	---	---	---	---	---	---	---
Late peri-/postmenopausal	0.140 (0.094)	0.138	-0.048 (0.060)	0.430	0.178 (0.128)	0.165	0.152 (0.146)	0.297
Hormone users	-0.271 (0.135)	**0.045**	0.098 (0.086)	0.254	-0.379 (0.182)	**0.039**	0.178 (0.208)	0.394
Education		0.081[*]		0.400[*]		0.080[*]		0.460[*]
≤ High school	---	---	---	---	---	---	---	---
Some college/ vocational school	0.159 (0.120)	0.188	-0.093 (0.076)	0.221	0.244 (0.163)	0.135	0.075 (0.184)	0.682
≥ College degree	0.283 (0.127)	**0.027**	-0.101 (0.080)	0.206	0.389 (0.172)	**0.025**	-0.108 (0.194)	0.578
Household Income		0.405[*]		0.064[*]		0.312[*]		0.057[*]
< $50k	---	---	---	---	---	---	---	---
≥ $50k to < $75k	0.146 (0.116)	0.207	0.056 (0.072)	0.443	0.072	0.647	0.086	0.624
≥ $75k	0.025 (0.102)	0.805	0.146 (0.063)	**0.022**	-0.140	0.309	-0.272	0.075
Financial Strain	0.023 (0.093)	0.805	-0.196 (0.058)	**<0.001**	0.212 (0.126)	0.094	0.081 (0.143)	0.571
Current Smoker	-0.201 (0.121)	0.096	-0.028 (0.077)	0.711	-0.179 (0.164)	0.276	0.076 (0.186)	0.684
Alcohol Consumption		**<0.001**[*]		**<0.001**[*]		**<0.001**[*]		**0.012**[*]
≤ 1/month	---	---	---	---	---	---	---	---
> 1/month to 1/week	-0.150 (0.096)	0.120	0.130 (0.061)	**0.032**	-0.265 (0.128)	**0.040**	-0.050 (0.148)	0.736
≥ 2/week	-0.496 (0.108)	**<0.001**	0.303 (0.069)	**<0.001**	-0.799 (0.145)	**<0.001**	-0.487 (0.170)	**0.004**
Physical Activity	-0.130 (0.025)	**<0.001**	0.092 (0.016)	**<0.001**	-0.218 (0.034)	**<0.001**	-0.138 (0.040)	**<0.001**
SBP, mmHg	0.016 (0.003)	**<0.001**	-0.0089 (0.002)	**<0.001**	0.025 (0.003)	**<0.001**	0.013 (0.004)	**0.002**
DBP, mmHg	0.024 (0.0040	**<0.001**	-0.012 (0.003)	**<0.001**	0.036 (0.006)	**<0.001**	0.015 (0.007)	**0.020**

Supplemental Table 5-2: Continued

Hypertension	0.506 (0.096)	<**0.001**	-0.272 (0.061)	<**0.001**	0.771 (0.129)	<**0.001**	0.419 (0.151)	**0.006**
HDL-C, mg/dL	-0.014 (0.003)	<**0.001**	0.014 (0.002)	<**0.001**	-0.028 (0.004)	<**0.001**	-0.018 (0.005)	<**0.001**
LDL-C, mg/dL	0.318 (0.168)	<**0.001**	-0.0068 (0.104)	<**0.001**	0.332 (0.227)	0.145	0.102 (0.260)	0.696
Triglycerides, mg/dL	0.387 (0.090)	<**0.001**	-0.257 (0.056)	<**0.001**	0.644 (0.120)	<**0.001**	0.482 (0.141)	<**0.001**
Cholesterol Medication	0.218 (0.169)	0.199	-0.185 (0.104)	0.078	0.463 (0.228)	**0.044**	0.313 (0.257)	0.223
BP/Cholesterol Medication	0.418 (0.101)	<**0.001**	-0.184 (0.064)	**0.004**	0.620 (0.136)	<**0.001**	0.293 (0.158)	0.064
BP Medication	0.432 (0.108)	<**0.001**	-0.214 (0.069)	**0.002**	0.639 (0.146)	<**0.001**	0.301 (0.1700	0.078
HOMA-IR	0.552 (0.065)	<**0.001**	-0.286 (0.043)	<**0.001**	0.837 (0.085)	<**0.001**	0.699 (0.109)	<**0.001**
Diabetes	0.172 (0.212)	0.419	-0.136 (0.134)	0.312	0.302 (0.288)	0.294	1.021 (0.325)	**0.002**
BMI, kg/m^2	0.085 (0.005)	<**0.001**	-0.027 (0.004)	<**0.001**	0.112 (0.007)	<**0.001**	0.087 (0.009)	<**0.001**
VAT, cm^2	0.887 (0.060)	<**0.001**	-0.302 (0.046)	<**0.001**	1.181 (0.083)	<**0.001**	0.851 (0.110)	<**0.001**
SAT, cm^2	0.0033 (0.00022)	<**0.001**	-0.0084 (0.00017)	<**0.001**	0.0041 (0.0030)	<**0.001**	0.0033 (0.00039)	<**0.001**
EAT, cm^3	0.656 (0.084)	<**0.001**	-0.227 (0.056)	<**0.001**	0.869 (0.115)	<**0.001**	0.800 (0.132)	<**0.001**
PAT,cm^3	0.515 (0.048)	<**0.001**	-0.139 (0.034)	<**0.001**	0.645 (0.067)	<**0.001**	0.489 (0.079)	<**0.001**
TAT, cm^3	0.734 (0.078)	<**0.001**	-0.232 (0.054)	<**0.001**	0.951 (0.108)	<**0.001**	0.836 (0.126)	<**0.001**

Data presented as linear regression beta coefficients (standard error); *global p-value; SBP, systolic blood pressure; DBP, diastolic blood pressure; BP/Cholesterol medication, taking blood pressure or cholesterol lowering medication; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; diabetes, defined as maximum glucose $\geq$ 126 or taking diabetes medication; hypertension, defined as SBP $\geq$ 140 or DBP $\geq$ 90 or taking blood pressure medication; HOMA-IR, homeostatic model assessment of insulin resistance; BMI, body mass index; VAT, abdominal visceral fat; SAT, abdominal subcutaneous fat; EAT, epicardial adipose tissue; PAT, paracardial adipose tissue; TAT, total heart adipose tissue; LA ratio, leptin to adiponectin ratio; CRP, high-sensitivity c-reactive protein; time interval, time in years between cardiovascular fat scan and adipokine/inflammatory marker measurements; EAT, PAT, TAT, LDL-C, triglycerides, HOMA-IR, leptin, adiponectin, LA ratio, and CRP were log-transformed

Supplemental Table 5-3: Characteristics of the study population overall and by CAC progression status

Variables	N	Total (N=222)	No CAC Progression (n=175/ 78.8%)	CAC Progression (n=47/ 21.2%)	P-value
Age, years	222	51.3 ± 2.7	51.2 ± 2.6	51.5 ± 2.9	0.586
Black, n (%)	222	76 (34.2)	59 (33.7)	17 (36.2)	0.753
Chicago Site, n (%)	222	100 (45.0)	78 (44.6)	22 (46.8)	0.784
Menopausal Status, n (%)	222				0.114*
Pre-/early peri-menopausal		126 (56.8)	100 (57.1)	26 (55.3)	
Late peri-/postmenopausal		70 (31.5)	51 (29.1)	19 (40.4)	
Hormone Users		26 (11.7)	24 (13.7)	2 (4.3)	
Education, n (%)	222				0.362*
≤ High school		39 (17.6)	30 (17.1)	9 (19.2)	
Some college/vocational school		112 (50.4)	85 (48.6)	27 (57.4)	
≥ College degree		71 (32.0)	60 (34.3)	11 (23.4)	
Household Income, n (%)	222				0.640*
< $50k		69 (31.2)	52 (29.7)	17 (37.0)	
≥ $50k to < $75k		57 (25.8)	46 (26.3)	11 (23.9)	
≥ $75k		95 (43.0)	77 (44.0)	18 (39.1)	
Financial Strain, n (%)	222	69 (31.1)	50 (28.6)	19 (40.4)	0.119
Current Smoker, n (%)	222	30 (13.5)	22 (12.6)	8 (17.0)	0.428
Alcohol Consumption	222				0.407*
≤ 1/month		96 (43.2)	75 (42.9)	21 (44.7)	
> 1/month to 1/week		83 (37.4)	63 (36.0)	20 (42.6)	
≥ 2/week		43 (19.4)	37 (21.1)	6 (12.8)	
Physical Activity	222	7.84 ± 1.7	7.90 ± 1.6	7.61 ± 1.8	0.282
SBP, mmHg	219	117.5 ± 15.2	116.3 ± 15.0	121.9 ± 15.3	**0.026**
DBP, mmHg	219	75.0 ± 9.2	74.4 ± 9.3	77.5 ± 8.5	**0.042**
Hypertension, n (%)	220	52 (23.6)	35 (20.1)	17 (37.0)	**0.017**
HOMA-IR	194	1.90 (1.45, 3.12)	1.81 (1.42, 3.10)	2.26 (1.53, 3.17)	0.447
Diabetes, n (%)	222	5 (2.2)	2 (1.1)	3 (6.4)	0.065†
HDL-C, mg/dL	208	56.9 ± 13.6	57.8 ± 14.0	53.8 ± 11.6	0.694
LDL-C, mg/dL	198	115.5 (96.0, 138.0)	114.0 (92.0, 136.0)	117.0 (102.0, 148.0)	0.094

Supplemental Table 5-3: Continued

Triglycerides, mg/dL	201	100.0 (77.0, 138.0)	99.0 (77.0, 136.5)	105.0 (76.0, 169.0)	**0.003**
Cholesterol Medication, n (%)	222	18 (8.1)	9 (5.1)	9 (19.2)	**0.004**
BP/Cholesterol Medication, n (%)	222	51 (23.0)	35 (20.0)	16 (34.0)	**0.042**
BP Medication, n (%)	222	42 (18.9)	29 (16.6)	13 (27.7)	0.085
BMI, kg/m^2	217	29.2 ± 6.2	28.6 ± 5.9	31.2 ± 6.8	**0.011**
VAT, cm^2	222	113.0 (70.4, 166.4)	100.6 (68.6, 157.9)	145.0 (107.0, 192.7)	<0.001
SAT, cm^2	222	341.4 ± 152.8	331.6 ± 151.0	378.0 ± 155.7	0.064
EAT, cm^3	222	36.3 (27.4, 51.7)	35.2 (26.7, 51.5)	40.9 (30.0, 57.2)	0.333
PAT, cm^3	222	8.49 (4.38, 14.51)	7.39 (4.32, 14.60)	10.44 (5.57, 13.83)	0.393
TAT, cm^3	222	44.1 (33.5, 64.9)	41.8 (33.5, 63.9)	53.8 (36.0, 70.9)	0.332
Leptin, ng/mL	214	29.2 (17.0, 43.5)	26.1 (16.4, 41.9)	36.3 (23.3, 45.6)	0.086
Adiponectin, µg/mL	215	12.11 (9.20, 16.47)	12.35 (9.53, 17.04)	10.47 (7.67, 13.93)	**0.004**
LA Ratio	214	2.44 (1.24, 4.33)	2.27 (1.13, 3.91)	3.20 (1.72, 5.39)	**0.010**
CRP, mg/dL	221	2.10 (1.00, 4.40)	1.95 (1.00, 4.60)	2.20 (0.90, 4.30)	0.856
Baseline CAC Score	222	0.00 (0.00, 6.18)	0.00 (0.00, 4.81)	0.00 (0.00, 31.24)	**0.006**
Time Interval, years	222	2.32 ± 0.48	2.31 ± 0.45	2.37 ± 0.58	0.492

*global p-value; †Fisher's exact test p-value; SBP, systolic blood pressure; DBP, diastolic blood pressure; BP/Cholesterol medication, taking blood pressure or cholesterol lowering medication; hypertension, SBP ≥140 or DBP ≥90 or taking blood pressure medication; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; diabetes, maximum glucose ≥126 or taking diabetes medication; HOMA-IR, homeostatic model assessment of insulin resistance; BMI, body mass index; VAT, abdominal visceral fat; SAT, abdominal subcutaneous fat; EAT, epicardial adipose tissue; PAT, paracardial adipose tissue; TAT, total heart adipose tissue; LA ratio, leptin to adiponectin ratio; CRP, high sensitivity c-reactive protein; time interval, time in years between baseline CAC and follow-up CAC scans; EAT, PAT, TAT, VAT, LDL-C, triglycerides, HOMA-IR, leptin, adiponectin, LA ratio, and CRP were log-transformed; note: cell numbers may not add up to the column total due to missing values for some of the variables.

Supplemental Table 5-4: Univariate associations between participant characteristics and measures of the extent of CAC progression

	Extent of CAC Progression	
Variables	β (s.e.)	p
Age, years	0.0041 (0.003)	0.144
Black	-0.0015 (0.016)	0.802
Chicago site	0.0039 (0.015)	0.918
Menopausal Status		0.071
Pre-/early peri-menopausal	---	---
Late peri-/postmenopausal	0.032 (0.016)	0.054
Hormone users	-0.018 (0.024)	0.451
Education		0.126
≤ High school	---	---
Some college/vocational school	-0.023 (0.020)	0.259
≥ College degree	-0.044 (0.022)	**0.045**
Household Income		0.503
< $50k	---	---
≥ $50k to < $75k	-0.011 (0.020)	0.566
≥ $75k	-0.020 (0.017)	0.242
Financial Strain	0.0094 (0.016)	0.558
Current Smoker, n (%)	0.022 (0.022)	0.314
Alcohol Consumption		0.357
≤ 1/month	---	---
> 1/month to 1/week	-0.021 (0.017)	0.211
≥ 2/week	-0.023 (0.020)	0.261
Physical Activity	-0.0037 (0.004)	0.413
SBP, mmHg	0.0012 (0.0005)	**0.018**
DBP, mmHg	0.0019 (0.0008)	**0.016**
Hypertension	0.057 (0.017)	**<0.001**
HDL-C, mg/dL	-0.00036 (0.0006)	0.534
LDL-C, mg/dL	0.019 (0.028)	0.513
Triglycerides, mg/dL	0.034 (0.016)	**0.035**
Cholesterol Medication	0.060 (0.027)	**0.026**
BP/Cholesterol Medication	0.041 (0.017)	**0.019**
BP Medication	0.039 (0.019)	**0.038**
HOMA-IR	0.0044 (0.013)	0.743
Diabetes	0.074 (0.050)	0.136
BMI, kg/m^2	0.0037 (0.001)	**0.002**
VAT, cm^2	0.047 (0.013)	**<0.001**
SAT, cm^2	0.00011 (0.00005)	**0.014**
EAT, cm^3	0.021 (0.015)	0.177
PAT,cm^3	0.015 (0.009)	0.107

Supplemental Table 5-4: Continued

TAT, cm^3	0.022 (0.015)	0.142
Leptin, ng/mL	0.023 (0.011)	**0.033**
Adiponectin, µg/mL	-0.029 (0.016)	0.080
LA Ratio	0.018 (0.008)	**0.018**
CRP, mg/dL	0.0051 (0.007)	0.452
Baseline CAC Score	0.014 (0.005)	**0.004**
Time Interval, years	0.015 (0.016)	0.340

Data presented as linear regression beta coefficients (standard error); *global p-value; SBP, systolic blood pressure; DBP, diastolic blood pressure; BP/Cholesterol medication, taking blood pressure or cholesterol lowering medication; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; diabetes, defined as maximum glucose $\geq$ 126 or taking diabetes medication; hypertension, defined as SBP $\geq$ 140 or DBP $\geq$ 90 or taking blood pressure medication; HOMA-IR, homeostatic model assessment of insulin resistance; BMI, body mass index; VAT, abdominal visceral fat; SAT, abdominal subcutaneous fat; EAT, epicardial adipose tissue; PAT, paracardial adipose tissue; TAT, total heart adipose tissue; LA ratio, leptin to adiponectin ratio; CRP, high sensitivity c-reactive protein; time interval, time in years between baseline and follow-up scans; EAT, PAT, TAT, LDL-C, triglycerides, HOMA-IR, leptin, adiponectin, LA ratio, and CRP were log-transformed; extent of CAC progression was defined using the following equation:
$[\log(CAC_{(follow\text{-}up)}+25) - \log(CAC_{(baseline)}+25)]$/time (years) between baseline and follow-up scans.

6.0 CARDIOVASCULAR FAT RADIODENSITY AND SUBCLINICAL ATHEROSCLEROSIS IN MIDLIFE WOMEN: THE SWAN CARDIOVASCULAR FAT ANCILLARY STUDY

6.1 ABSTRACT

Background: Adipose tissue radiodensity as measured by CT Hounsfield units (HU) is a potential marker of fat quality. We sought to determine the cross-sectional associations of total heart fat (TAT) and aortic perivascular fat (PVAT) radiodensity with cardiovascular risk factors, coronary artery calcification (CAC), and aortic calcification (AC) in midlife women. **Methods and Results**: TAT and PVAT radiodensity values as well as CAC (CAC presence: Agatston score $\geq$10) and AC (AC presence: Agatston score $\geq$100) were quantified using CT scans. A total of 528 women (mean age: 50.9 years; 37% Black; and 34% late peri-/postmenopausal) were included in the analyses. Women in the lowest TAT radiodensity tertile were significantly more likely to be White and to have adverse cardiovascular risk factors. Results were less pronounced for PVAT radiodensity tertiles. Independent of cardiovascular risk factors, women in the middle and high TAT radiodensity tertiles were less likely to have CAC (OR 0.32; 95% CI (0.18, 0.59); OR 0.43; 95% CI (0.24, 0.78); respectively). Although adjusting for BMI attenuated the overall association, women in the middle TAT radiodensity tertile remained at significantly lower odds of CAC when compared to women in the low radiodensity tertile, p-value = 0.03. Associations were not significant between PVAT radiodensity tertiles and AC presence in the final models. **Conclusions**: Lower TAT radiodensity was associated with a less favorable cardiometabolic profile; however the results were not as clear for CAC and AC. More research is necessary to understand radiodensity as a surrogate marker of fat quality in midlife women

6.2 INTRODUCTION

Adipose tissue is a complex and metabolically active organ with potential endocrine, paracrine, and vasocrine influences that convey differing cardiovascular risk depending on the location in the body.[5,62,71,83,182] The quantities of cardiovascular fat, defined as the fat around the heart and the vasculature, are predictors of subclinical atherosclerosis measures (e.g. coronary (CAC) and aortic calcification (AC)) and cardiovascular disease (CVD) independent of other measures of adiposity, such as body mass index (BMI), waist circumference, or abdominal visceral fat (VAT).[14,83,182] Atherosclerotic calcification is a progressive condition and an indicator of the extent of atherosclerotic disease evolution.[129,215] CAC strongly predicts future CVD events and mortality; while AC has been shown to predict all-cause mortality.[151,152,159] Recently, we found that postmenopausal women have higher volumes of cardiovascular fat around the heart when compared to premenopausal women, independent of age, obesity, and other potential risk factors.[52] It is possible that higher volumes of cardiovascular fat found in postmenopausal women may, in part, contribute to the increased cardiovascular risk reported after menopause.[17,18,52]

Most recently, attention has been focused on the quality of cardiovascular fat as a novel marker of CVD risk.[9,37,45,50] Adipose tissue quality characteristics, such as adipocyte hypertrophy and hyperplasia, adipocyte hypoxia, macrophage accumulation, capillary density, and type of adipocytes have been evaluated, and evidence suggests associations between these fat quality parameters and cardiovascular and metabolic risks.[38-43] Due to the invasive nature of the procedures used to assess these fat quality characteristics, limited research is available.[38-40] Fat

radiodensity measured by computed tomography (CT) Hounsfield units (HU), has been proposed as a surrogate marker of adipose tissue quality.[9,45] Higher adipose tissue radiodensity may indicate adipocytes that are densely packed with mitochondria and multiple lipid droplets, higher levels of vascularization and innervation, and fewer hypertrophic adipocytes.[46,47] In contrast, lower fat radiodensity may indicate hypertrophic adipocytes and higher levels of lipid content that may be more harmful and represent a lower quality adipose tissue.[9,40,46] Only a few studies have evaluated fat radiodensity as a risk factor for CVD and the results appear to be inconsistent. Studies have found positive, negative, and no associations between epicardial fat (EAT), VAT, and subcutaneous fat (SAT) radiodensity and CVD risk factors, events, and adipokines.[9,37,45,50,185]

To the best of our knowledge, no studies have evaluated the associations of total heart adipose tissue (TAT: fat inside and outside the pericardium) and aortic perivascular adipose tissue (PVAT: fat along the descending aorta) radiodensity values as related to CVD risk factors and subclinical measures of atherosclerosis in midlife women. Evaluating cardiovascular fat radiodensity as a surrogate marker of fat quality among midlife women may help to elucidate possible mechanisms for the higher rates of CVD seen among postmenopausal women.[17,18] Therefore, the objectives of our study were to determine the cross-sectional associations of TAT and PVAT radiodensity values with CVD risk factors, CAC, and AC in women at midlife, a time period marked with a higher CVD risk among women.[17,18,216] We hypothesized that women with higher TAT and PVAT radiodensity values would have lower odds of both CAC and AC presence and a more favorable cardiovascular risk profile.

6.3 METHODS

Study Population

The Study of Women's Health Across the Nation (SWAN) is a multicenter, community-based prospective study of women transitioning through menopause. The study design and objectives have been previously reported.[194] Briefly, 3302 participants aged 42-52 years were recruited from seven sites (Boston, MA; Detroit, MI; Oakland, CA; Los Angeles, CA; Pittsburgh, PA; Chicago, IL; and Newark, NJ) between 1996 and 1997.[52,194] In order to be eligible, women needed to have an intact uterus, to have at least one ovary, to have at least one menstrual period within the past 3 months, and to have not been on hormone therapy within the past 3 months.[52] The SWAN Heart ancillary study was designed to measure subclinical atherosclerosis among White and Black women at the Pittsburgh and Chicago study sites.[52] The SWAN Cardiovascular Fat ancillary study was designed to quantify volumes of cardiovascular fat depots among SWAN Heart participants using previously attained CT images.[52] A total of 564 out of the 608 SWAN Heart participants who had CT scans were included in the SWAN Cardiovascular Fat ancillary study of which 562 had readable PVAT or TAT measures.

For the current analyses, participants were excluded if they were missing both AC and CAC readings (n=10), had a history of stroke, angina, or heart attack (n=11), or had undergone surgical menopause (n=13). A total of 528 women were included in the PVAT analyses. Due to either poor image quality or scans that did not encompass the designated anatomical boundaries for the TAT depot an additional 40 participants were excluded from TAT analyses, leaving a

total of 488 women. The institutional review board at each site approved the study protocol and all participants signed informed consent prior to participation.

CAC and AC Quantification

Calcification of the coronary arteries and descending and abdominal aorta were measured using electron-beam CT with an Imatron C-150 Ultrafast scanner (GE-Imatron, South San Francisco, CA) at each site. Three passes were performed. The first scan was a scout pass to determine anatomical landmarks. The second pass provided 30 to 40 contiguous 3-mm-thick transverse images of the coronary arteries from the level of the aortic root to the apex of the heart. Images were taken during maximal breath holding using electrocardiographic triggering to obtain 100-ms exposures during the same phase of the cardiac cycle (60% of the RR interval). The third pass provided cross-sectional 6-mm images of the aorta from the aortic arch to the iliac bifurcation. Each image was taken with a 300-ms exposure time during maximal breath holding. Scans were scored according to the Agatston method using a DICOM workstation and AccuImage, Inc. software (South San Francisco, CA) at the University of Pittsburgh.[135] Calcification was considered present if there were at least 3 contiguous pixels with a radiodensity $\geq$130 HU. Agatston scores were individually calculated for CAC and AC. CAC was quantified for each of the 4 major coronary arteries and then summed for a total Agatston score; while AC was quantified as a single total Agatston score. Excellent reproducibility has been reported for the CAC and AC quantification protocols with intra-class correlation coefficients of 0.99 for CAC and 0.98 for AC.[207]

Cardiovascular Fat Depot Quantification

As previously described, TAT was defined as the combination of the fat inside and outside the pericardium.[52] TAT radiodensity and volume were quantified at the Los Angeles Biomedical Research Institute, Harbor-UCLA Medical Center, CA, USA, using the same set of images acquired during the CAC electron-beam CT scanning. TAT volumes were determined from 15 mm above to 30 mm below the superior extent of the left main coronary artery. Using volume analysis software (GE Healthcare, Waukesha, WI, USA), adipose tissue was distinguished from the remainder of the heart tissue by a threshold of -190 to -30 HU. The aorta and bronchus defined the posterior border, while the chest wall defined the anterior border. Posterior mediastinum and peri-aortic adipose tissues were not included. TAT was measured by using a semi-automated method with the technician manually tracing the area of interest every 2-3 CT slices, while the software automatically traced the segments in between these selected slices. Excellent within- and between-reader spearmen correlation coefficients of 0.97 and 0.97 have been reported for this fat quantification protocol.[52] TAT radiodensity was quantified as a single mean radiodensity value for all CT images read.

PVAT was quantified at the Ultrasound Research Lab at the University of Pittsburgh using the same set of images acquired during the AC electron-beam CT scanning. PVAT was defined as the adipose tissue immediately surrounding the descending aorta. Using a dedicated image analysis workstation equipped with Slice-O-Matic v4.3 (TomoVision, Magog, Quebec, Canada), adipose tissue was distinguished from other tissues by a threshold of -190 to -30 HU. The pulmonary bifurcation marked the proximal border and the first lumbar vertebrae served as the distal border. The anterior borders included the left bronchus, esophagus, and eventually the interior border of the crus of the diaphragm; while the anterior border of the vertebral foramen

served as the posterior border. The borders of the area of interest were manually traced for every slice. A similar protocol has been used before with excellent intra-reader and inter-reader reproducibility for this measure (intra-class coefficient 0.999 and 0.998, respectively).[83] PVAT radiodensity was quantified as a single mean radiodensity value for all CT images read.

Study Covariates

Weight and height were measured in light clothing and without shoes. Weight was measured using a standardized, calibrated scale and height was measured using a stadiometer. BMI was calculated as weight in kilograms divided by height in square meters. After at least 5 minutes of rest, blood pressure was measured in the right arm with the participant seated, using a mercury sphygmomanometer. Blood pressure readings were taken twice and averaged. Race, age, financial strain, and current smoking status were self-reported. Financial strain was derived from the interview question, "How hard is it for you to pay for the very basics like food, housing, medical care, and heating?" For analyses, the answers were dichotomized as "somewhat hard to very hard" and "not hard at all".

Menopausal status was determined using self-reported bleeding patterns and categorized as follows: 1) premenopausal: menses in the last 3 months with no change in regularity in the last 12 months; 2) early peri-menopausal: menses in the last 3 months with some change in regularity during the prior 12 months; 3) late peri-menopausal: no menses within the last 3 months, but some menstrual bleeding over the prior 12 months; and 4) postmenopausal: no menses with the last 12 months. Pre- or peri-menopausal women who reported taking hormone therapy in the past year were considered indeterminate status as hormone use could potentially impact bleeding patterns and thus lead to a misclassification. On the other hand, postmenopausal women who

reported hormone therapy use were classified as hormone therapy users since their menopausal status was identified before being on hormone therapy. For the current analyses, the following menopausal categories were created similar to previous reports from SWAN to account for small sample sizes in certain menopausal and hormone therapy use categories: premenopausal and early peri-menopausal were combined into one group; late peri-menopausal and postmenopausal were combined into a second group; and the categories of indeterminate status and hormone therapy users were combined into a third group.[52,197]

CVD risk factors were assayed at the Medical Research Laboratories (Lexington, KY, USA), which is certified by the National Heart, Lung, and Blood Institute, Centers for Disease Control Part III program, as previously described.[208] Triglyceride levels were analyzed using enzymatic methods on a Hitachi 747 analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN, USA). High-density lipoprotein cholesterol (HDL-C) was isolated using heparin-2M manganese chloride. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation.[209] Serum insulin was measured using a radioimmunoassay (RIA; DPC Coat-a-count, Los Angeles, CA, USA) procedure. Glucose was measured using a hexokinase-coupled reaction (Boehringer Mannheim Diagnostics). The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated from insulin and glucose as (fasting insulin (mU/L) x fasting glucose (mmoles/L))/22.5.[210]

Statistical Analyses

Participant characteristics were summarized and presented as mean ± standard deviation for normally distributed variables; median (interquartile range) for skewed variables; and frequency (percentage) for categorical variables. Normality was assessed for all continuous

variables. Triglycerides, PVAT and TAT volumes, HOMA-IR, and LDL-C were log-transformed. The presence of CAC was defined as an Agatston score of greater than or equal to 10 and the presence of AC was defined as an Agatston score of greater than or equal to 100.[217,218] Cardiovascular fat radiodensity values were first analyzed as continuous variables. These preliminary analyses suggested a nonlinear effect. Therefore, the main analyses reported in the current manuscript utilized tertiles of radiodensity for each fat depot. Chi-square and analysis of variance tests were used to determine whether participant characteristics and traditional CVD risk factors varied across tertiles of both TAT and PVAT radiodensity. Trends were assessed across tertiles of cardiovascular fat radiodensity for continuous variables.

Separate logistic regression models were created to assess the relationships between the tertiles of radiodensity for each cardiovascular fat depot (TAT and PVAT, separate models) as the independent variable and the presence of CAC and AC in separate models as the dependent variable. To build the best fitting parsimonious model, backward elimination model-building procedures were used to select potential covariates. Age, study site, race, menopausal status, and BMI were a priori selected covariates and were forced into the model regardless of statistical significance. All p-values less than 0.05 were considered to be statistically significant for all analyses. Interactions between cardiovascular fat radiodensity and race and menopausal status were individually assessed in each model. Given that previous studies adjusted for cardiovascular fat volume, we assessed the correlations between the radiodensity and volume for each depot. Adjusting for these fat volumes made our models unstable; therefore, we explored the associations between cardiovascular fat radiodensity and calcification stratified by tertiles of cardiovascular fat volume (**Supplemental Tables 6-1 and 6-2)**. Due to the small cell sizes, the

adjusted model for these exploratory volume-stratified analyses only included the following a priori selected covariates: age, race, study site, menopausal status, and BMI.

To compare our results with other studies, we conducted exploratory analyses looking at EAT radiodensity (**Supplemental Table 6-3**). In addition, because the majority (56%) of Black women were in the highest TAT radiodensity tertile and only 10% were in the lowest radiodensity tertile, we explored race-stratified analyses (**Supplemental Tables 6-4 and 6-5**). The covariates in the adjusted model included the same variables in the fully-adjusted models for the primary analyses (model 4: age, race, study site, menopausal status, BMI, log-transformed triglycerides, current smoking status, and systolic blood pressure). All analyses were conducted using SAS v9.3 (SAS Institute, Cary, North Carolina).

6.4 RESULTS

Cardiovascular Risk Factors Across Cardiovascular Fat Radiodensity Tertiles

The characteristics of the participants by tertiles of TAT and PVAT radiodensity are presented in **Tables 6-1 and 6-2**. The radiodensity values ranged from -91.0 HU to -67.0 HU for TAT and ranged from -95.2 HU to -68.3 HU for PVAT. Women in the lowest TAT radiodensity tertile were significantly more likely to be White and to have higher LDL-C, triglycerides, HOMA-IR, BMI, TAT volume, and PVAT volume, and lower HDL-C. They were also more likely to have CAC and AC present (30% and 33%, respectively). Women in the lowest PVAT radiodensity tertile were significantly more likely to be White, have lower HDL-C, and have higher triglycerides and PVAT volume. No other differences including the presence of CAC or AC were found across PVAT tertiles.

TAT Radiodensity and Subclinical Atherosclerosis

The odds for the presence of CAC and AC by TAT radiodensity tertiles derived from multivariable logistic regression are shown in **Table 6-3**. When compared to the low TAT radiodensity tertile, the odds of having CAC were significantly lower for the middle and high radiodensity tertiles in the partially adjusted model (model 2). After further adjustment for BMI (model 3), the overall association was attenuated; however, the odds for having CAC remained significantly lower for the middle radiodensity tertile compared to the lowest tertile. In the final model (model 4), the results remained consistent. The odds of having AC were significantly lower for the high TAT radiodensity tertiles in model 3 when compared to the low radiodensity

tertile. In the final model (model 4), the results were attenuated and no longer significant. There were no significant interactions between TAT radiodensity and race or menopausal status for the presence of either subclinical atherosclerosis measure.

PVAT Radiodensity and Subclinical Atherosclerosis

The associations between PVAT radiodensity and the presence of CAC and AC using multivariable logistic regression are shown in **Table 6-4**. No associations between PVAT radiodensity and the presence of CAC were found in any of the models. The odds of the presence of AC were significantly lower for the high radiodensity tertile when compared to the low tertile in model 2. After additional adjustment for BMI (model 3), the results were attenuated and no longer significant. There were no significant interactions between PVAT radiodensity and race or menopausal status for the presence of either subclinical atherosclerosis measure.

Exploratory Analyses

In analyses stratified by TAT volume, significant associations between TAT radiodensity and the presence of CAC and AC were evident only in the highest TAT volume stratum (**Supplemental Table 6-1**). In the high TAT volume strata, the middle TAT radiodensity tertile was inversely associated with the presence of CAC and AC in the unadjusted and adjusted models. No significant associations between PVAT radiodensity and subclinical atherosclerosis measures were found for PVAT volume stratified analyses (**Supplemental Table 6-2**).

In the exploratory analyses assessing EAT, no associations were found between EAT radiodensity and the presence of CAC in any of the models (**Supplemental Table 6-3**). For the

presence of AC, the highest EAT radiodensity was inversely associated with AC presence in the partially adjusted model (model 2); however, after adjusting for BMI the associations were attenuated and no longer significant (model 3).

In race-stratified analyses, there does not appear to be an evident racial difference in the association between TAT radiodensity and CAC (**Supplemental Table 6-4**). However, we found that the odds of having AC were significantly lower for those in the highest TAT radiodensity tertile when compared to the lowest tertile in our adjusted model among White women, but not Black women. For PVAT radiodensity, no racial differences were found for either subclinical atherosclerosis measure (**Supplemental Table 6-5**).

6.5 DISCUSSION

Our cross-sectional study among women at midlife has three main findings. First, TAT radiodensity was associated with several CVD risk factors, such that women in the lowest radiodensity tertile had a less favorable cardiovascular profile. Second, women with mid-range TAT radiodensity values had a lower odds of CAC presence, independent of CVD risk factors and BMI. Third, we did not find any significant associations between PVAT radiodensity and CAC or AC, and the associations of PVAT radiodensity with CVD risk factors were less pronounced.

Our study provides a look at the radiodensity of cardiovascular fat depots that have not been assessed previously in midlife women. We found a marked difference in several CVD risk factors across tertiles of TAT radiodensity with higher radiodensity values being more favorable. Interestingly, only HDL-C and triglycerides were significantly associated with both TAT and PVAT radiodensity values. Consistent with our study, researchers found that higher radiodensity values for VAT and SAT depots were associated with more favorable cardiometabolic and adipokine profiles, among Framingham Heart Study MDCT substudy participants.[9,219] In contrast, our findings of the lower odds of CAC presence for higher TAT radiodensity values compared to lower radiodensity values was not consistent with current literature. Only two studies have published findings regarding the associations between fat radiodensity and subclinical atherosclerosis. Pracon et al. found that higher EAT radiodensity values were positively correlated with CAC severity among participants suspected of coronary artery disease in minimally adjusted analyses.[37] Among Framingham Heart Study MDCT substudy

participants, Alvey et al. found that higher VAT radiodensity values were positively associated with both CAC and abdominal aortic calcification; higher SAT radiodensity values were positively associated only with CAC; and EAT radiodensity was not associated with either measure of subclinical atherosclerosis.[45] Our findings were consistent with the results from Alvey et al. only in our exploratory analyses of EAT radiodensity where we found no association with CAC.

There are three potential factors that may have contributed to the differences between our studies. First, our studies evaluated different fat depots. We evaluated TAT which includes fat inside and outside the pericardium; while these studies looked at fat within the pericardium (EAT) and abdominal fat (VAT and SAT). Growing evidence suggests that these fat depots may differ in several adipocyte characteristics, such as metabolic activity and embryonic origin.[21,22] Second, our subclinical atherosclerosis measures differed in categorization and arterial bed. We defined the presence of CAC as an Agatston score of greater than or equal to 10; while the other studies used a higher Agatston score cut point of 100 or assessed CAC as an untransformed continuous variable.[37,45] In addition, we measured calcification along different portions of the thoracic and abdominal aorta. Third, our study population included Black and White women, while the other studies included both men and women in a White population.

We hypothesized that lower cardiovascular fat radiodensity would be associated with a less favorable cardiovascular profile for both fat depots. This hypothesis was based on current literature suggesting that lower radiodensity may represent hypertrophic adipocytes filled with lipids that are thought to secrete higher amounts of pro-inflammatory substances and increased levels of free fatty acids.[21,47,48,92] In addition, as adipose tissue expands beyond a certain threshold, angiogenesis may be incapable of keeping up causing hypoxic areas within the fat

depot.[66] Some studies have shown that capillary density and blood flow are reduced in obesity and that hypertrophic adipocytes may increase to a size too large for oxygen to diffuse the distance to the adipocyte mitochondria, causing necrosis.[39,41,66,220] This hypoxic state incites an inflammatory response, macrophage accumulation, and may contribute to insulin resistance and other adverse cardiometabolic characteristics.[41,66,220,221] To further complicate our understanding of fat radiodensity, adipose tissue with higher lipid content, hypertrophic cells, and lower capillary density, which could be characteristics of low radiodensity, may promote adipose tissue fibrosis.[39,46,66,220] Fibrosis may cause adipose tissue dysfunction and insulin resistance.[220,222] Fibrotic adipose tissue would be represented by higher radiodensity values and could possibly convolute the associations between fat radiodensity and atherosclerosis.[45] It is possible that this fibrotic condition contributed to the unexpected nonlinear effect with the mid-range radiodensity values, instead of the high radiodensity values, having the lowest odds of CAC presence in our study.

Strengths and Limitations

Our study has some limitations including the cross-sectional observational study design which prevented us from assessing temporality and introduced the possibility of unmeasured or residual confounders. Our study was limited to midlife women of Black or White race and, therefore, our findings may not be generalizable to men, younger women, or women from other racial/ethnic groups. AC was quantified along the entire descending thoracic and abdominal aorta, while PVAT mainly included the fat along the descending thoracic aorta. It is unclear whether this contributed to our null findings between PVAT radiodensity and AC. Only one mean HU value to represent the radiodensity of the entire fat depot may not provide a

comprehensive description of the distribution of higher and lower radiodensity fat. In addition, although CT radiodensity has been suggested as a surrogate marker of fat quality, research is still in the early stages of determining what the HU of fat represents. Higher radiodensity values may include high quality fat for some women and fibrotic tissue, and thus, a poorer fat quality for other women. Due to the high correlation between fat volume and fat radiodensity, adding fat volume into our models made them unstable. Although we conducted volume stratified analyses, we did not have a large enough sample size to adequately explore these associations. Lastly, because of the way TAT and EAT were quantified, we did not have the radiodensity of PAT available for evaluation. In previous analyses from our study, reductions in estradiol over a 4.8 year period was significantly associated with PAT volume but not EAT or TAT, indicating that estradiol may be especially important for this particular cardiovascular fat depot.[52] Since we found no association between EAT and CAC and because TAT is a combination of EAT and PAT, we suspect that our associations between TAT and CAC may be driven by PAT. Further analyses should evaluate PAT as a separate cardiovascular fat depot. This study has several strengths. It is the first study to evaluate surrogate markers of fat quality, measured via CT HU, for TAT and PVAT depots. No other studies have evaluated these cardiovascular fat depots in either men or women. We were able to utilize CVD risk factors, menopausal status, and subclinical atherosclerosis through the SWAN parent study, which is a well-established community-based study.

6.6 CONCLUSIONS

In conclusion, we found that midlife women with lower TAT and PVAT radiodensity values tended to have a less favorable cardiovascular profile, including associations with subclinical atherosclerosis. TAT was more strongly associated with CVD risk factors and subclinical atherosclerosis, than PVAT. Future studies with a larger sample size should evaluate these associations across strata of fat volume and by race/ethnicity. Evaluating PAT radiodensity separately may help further our understanding of the potential association between cardiovascular fat radiodensity and CVD risk. In addition, longitudinal studies evaluating the change in radiodensity may help to determine if low radiodensity fat becomes fibrotic over time.

6.7 TABLES AND FIGURES

Table 6-1: Characteristics of the study population across tertiles of TAT radiodensity

	TAT Radiodensity Tertiles (N=488)			
Characteristics	-91 to -81[†] Low (n=154)	-80 to -78[†] Middle (n=163)	-77 to -67[†] High (n=178)	P-value
Age (years)	51.4 ± 3.0	50.7 ± 3.0	50.9 ± 2.7	0.130[*]
Whites, n (%)	126 (83)	110 (68)	70 (40)	**<0.001**
Menopausal Status				0.064
Pre-/early peri-menopausal	71 (47)	91 (56)	107 (61)	
Late peri-/postmenopausal	66 (43)	57 (35)	49 (28)	
Hormone Users	15 (10)	14 (9)	18 (10)	
Smoking	31 (20)	33 (20)	24 (14)	0.193
Financial Strain	44 (31)	43 (28)	60 (36)	0.322
SBP (mmHg)	119.0 ± 15.7	118.5 ± 14.9	121.6 ± 17.7	0.143[*]
DBP (mmHg)	75.6 ± 9.5	75.8 ± 9.9	76.8 ± 10.4	0.278[*]
LDL-C (mg/dL)	120.0 (100.0, 148.0)	117.0 (97.0, 141.0)	110.0 (95.0, 131.5)	**0.020[*]**
HDL-C (mg/dL)	54.2 ± 13.4	59.3 ± 14.6	58.2 ± 15.0	**0.019[*]**
Triglycerides (mg/dL)	120.0 (87.0, 174.0)	98.5 (76.0, 136.8)	85.0 (68.0, 110.0)	**<0.001[*]**
HOMA-IR	2.3 (1.7, 3.9)	1.9 (1.4, 2.8)	1.9 (1.4, 3.1)	**0.042[*]**
BMI (kg/m^2)	30.8 ± 6.3	28.0 ± 5.4	29.0 ± 6.7	**0.016[*]**
TAT, cm^3	66.3 (51.5, 93.5)	44.3 (33.5, 59.7)	36.7 (29.6, 47.8)	**<0.001[*]**
PVAT, cm^3	37.3 (29.6, 45.1)	29.1 (23.9, 36.5)	26.9 (21.8, 32.8)	**<0.001[*]**
CAC Score	2.8 (0.0, 13.7)	0.0 (0.0, 3.1)	0.0 (0.0, 4.5)	**0.002[*]**
CAC $\geq$10	46 (30)	21 (13)	33 (19)	**<0.001**
AC Score	31.5 (2.4, 168.0)	6.1 (0.0, 66.0)	8.3 (0.0, 45.0)	**<0.001[*]**
AC $\geq$100	50 (33)	30 (19)	27 (16)	**<0.001**

[†]Hounsfield unit range of values; [*]p for trend; mean ± standard deviation; median (interquartile range); n (%); TAT, total heart adipose tissue; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; BMI, body mass index; CAC, coronary artery calcium; AC, aortic calcification; note: cell numbers may not add up to the column total due to missing values for some of the variables.

Table 6-2: Characteristics of the study population across tertiles of PVAT radiodensity

	PVAT Radiodensity Tertiles (N=528)			
Characteristics	-95.15 to -84.70† Low (n=183)	-84.68 to -82.02† Middle (n=174)	-82.01 to -68.26† High (n=179)	P-value
Age (years)	51.1 ± 3.1	50.8 ± 2.8	50.9 ± 2.8	0.588*
Whites, n (%)	129 (72)	103 (60)	99 (56)	**0.005**
Menopausal Status				0.822
Pre-/early peri-menopausal	100 (56)	100 (58)	95 (54)	
Late peri-/postmenopausal	63 (35)	57 (33)	61 (34)	
Hormone Users	16 (11)	15 (9)	21 (12)	
Smoking	33 (18)	36 (21)	23 (13)	0.135
Financial Strain	53 (31)	51 (31)	58 (34)	0.851
SBP (mmHg)	119.4 ± 14.8	118.5 ± 17.5	121.1 ± 17.5	0.365*
DBP (mmHg)	75.8 ± 9.5	75.6 ± 9.8	76.8 ± 10.7	0.370*
LDL-C (mg/dL)	113.0 (95.0, 140.0)	114.0 (98.0, 133.5)	117.0(97.0, 141.0)	0.467*
HDL-C (mg/dL)	54.2 ± 13.6	57.7 ± 15.0	59.7 ± 14.2	**<0.001***
Triglycerides (mg/dL)	109.0 (79.0, 156.0)	95.0 (73.0, 128.0)	92.0 (71.0, 125.0)	**<0.001***
HOMA-IR	2.1 (1.6, 3.9)	1.9 (1.3, 3.2)	2.0 (1.5, 2.8)	0.162*
BMI (kg/m^2)	30.1 ± 6.5	28.4 ± 6.4	29.1 ± 5.7	0.132*
TAT, cm^3	51.7 (35.3, 71.5)	45.3 (33.9, 59.8)	46.5 (35.0, 65.9)	0.063*
PVAT, cm^3	34.2 (25.0, 44.5)	28.8 (23.4, 37.2)	29.2 (24.0, 35.6)	**<0.001***
CAC Score	0.0 (0.0, 7.9)	0.0 (0.0, 6.4)	1.7 (0.0, 7.9)	0.912*
CAC $\geq$10	42 (23)	38 (22)	35 (20)	0.696
AC Score	21.0 (0.0, 138.0)	6.7 (0.0, 69.0)	16.0 (0.0, 63.0)	0.210*
AC $\geq$100	51 (29)	36 (21)	32 (18)	**0.049**

†Hounsfield unit range of values; *p for trend; mean ± standard deviation; median (interquartile range); n (%); PVAT, aortic perivascular adipose tissue; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; BMI, body mass index; CAC, coronary artery calcium; AC, aortic calcification; note: cell numbers may not add up to the column total due to missing values for some of the variables.

Table 6-3: Multivariable logistic regression for the associations between TAT radiodensity tertiles and the presence of CAC ≥10 and AC ≥100

CAC ≥10 Presence (n=488)			**AC ≥100 Presence** (n=486)		
TAT Radiodensity Tertiles	**OR (95% CI)**	**P-value**	**TAT Radiodensity Tertiles**	**OR (95% CI)**	**P-value**
Model 1		**<0.001***	**Model 1**		**<0.001***
Middle vs Low	0.34 (0.19, 0.61)	**<0.001**	Middle vs Low	0.46 (0.27, 0.77)	**0.003**
High vs Low	0.54 (0.32, 0.90)	**0.018**	High vs Low	0.38 (0.23, 0.65)	**<0.001**
Model 2		**<0.001***	**Model 2**		**<0.001***
Middle vs Low	0.32 (0.18, 0.59)	**<0.001**	Middle vs Low	0.45 (0.26, 0.78)	**0.004**
High vs Low	0.43 (0.24, 0.78)	**0.006**	High vs Low	0.33 (0.18, 0.60)	**<0.001**
Model 3		0.091*	**Model 3**		**0.017***
Middle vs Low	0.47 (0.24, 0.93)	**0.029**	Middle vs Low	0.56 (0.32, 0.99)	0.050
High vs Low	0.66 (0.34, 1.31)	0.238	High vs Low	0.42 (0.22, 0.72)	**0.006**
Model 4		0.084*	**Model 4**		0.116*
Middle vs Low	0.46 (0.23, 0.91)	**0.027**	Middle vs Low	0.59 (0.32, 1.09)	0.098
High vs Low	0.67 (0.33, 1.36)	0.264	High vs Low	0.52 (0.26, 1.03)	0.062

*global p-value for chi-square test with 2 degrees of freedom; TAT, total heart adipose tissue; CAC, coronary artery calcification; AC, aortic calcification; Model 1: unadjusted; Model 2: adjusted for age, race, site, menopausal status; Model 3: model 2 + body mass index; Model 4: model 3 + log-transformed triglycerides, current smoking status, and systolic blood pressure.

Table 6-4: Multivariable logistic regression for the associations between PVAT radiodensity tertiles and the presence of CAC ≥10 and AC ≥100

CAC ≥10 Presence (n=528)			**AC ≥100 Presence** (n=526)		
PVAT Radiodensity Tertiles	**OR (95% CI)**	**P-value**	**PVAT Radiodensity Tertiles**	**OR (95% CI)**	**P-value**
Model 1		0.696*	**Model 1**		0.051*
Middle vs Low	0.92 (0.56, 1.52)	0.760	Middle vs Low	0.66 (0.41, 1.08)	0.102
High vs Low	0.80 (0.48, 1.33)	0.398	High vs Low	0.55 (0.33, 0.91)	**0.020**
Model 2		0.542*	**Model 2**		**0.038***
Middle vs Low	0.91 (0.54, 1.54)	0.732	Middle vs Low	0.65 (0.39, 1.08)	0.096
High vs Low	0.74 (0.44, 1.27)	0.277	High vs Low	0.52 (0.31, 0.87)	**0.013**
Model 3		0.553*	**Model 3**		0.172*
Middle vs Low	1.35 (0.74, 2.48)	0.329	Middle vs Low	0.78 (0.46, 1.33)	0.365
High vs Low	1.03 (0.56, 1.87)	0.928	High vs Low	0.60 (0.35, 1.02)	0.061
Model 4		0.558*	**Model 4**		0.525*
Middle vs Low	1.40 (0.73, 2.66)	0.312	Middle vs Low	0.75 (0.42, 1.37)	0.356
High vs Low	1.06 (0.56, 2.02)	0.849	High vs Low	0.73 (0.40, 1.33)	0.311

*global p-value for chi-square test with 2 degrees of freedom; PVAT, aortic perivascular adipose tissue; CAC, coronary artery calcification; AC, aortic calcification; Model 1: unadjusted; Model 2: adjusted for age, race, site, menopausal status; Model 3: model 2 + body mass index; Model 4: model 3 + log-transformed triglycerides, current smoking status, and systolic blood pressure.

Supplemental Table 6-1: Multivariable logistic regression for the associations between TAT radiodensity and the presence of CAC ≥10 and AC ≥100 stratified by TAT volume

CAC ≥10 Presence						
	Low TAT Volume (n=161)		**Middle TAT Volume (n=162)**		**High TAT Volume (n=165)**	
TAT Radiodensity Tertiles	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
Unadjusted Model		0.758*		0.310*		0.051*
Middle vs Low	0.68 (0.06, 7.11)	0.747	0.96 (0.31, 2.98)	0.938	0.41 (0.18, 0.92)	**0.032**
High vs Low	1.14 (0.13, 9.96)	0.904	1.87 (0.65, 5.40)	0.247	1.32 (0.55, 3.21)	0.532
Adjusted Model[†]		0.895*		0.901*		0.053*
Middle vs Low	0.60 (0.05, 6.96)	0.681	0.78 (0.22, 2.77)	0.702	0.28 (0.10, 0.78)	**0.015**
High vs Low	0.78 (0.07, 8.25)	0.839	1.00 (0.26, 3.87)	0.996	0.60 (0.16, 2.26)	0.449
AC ≥100 Presence						
	Low TAT Volume (n=161)		**Middle TAT Volume (n=162)**		**High TAT Volume (n=165)**	
TAT Radiodensity Tertiles	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
Unadjusted Model		0.484*		0.105*		**0.028***
Middle vs Low	0.40 (0.08, 1.87)	0.244	1.38 (0.55, 3.50)	0.494	0.31 (0.13, 0.75)	**0.009**
High vs Low	0.45 (0.11, 1.90)	0.341	0.46 (0.15, 1.40)	0.171	0.97 (0.39, 2.38)	0.938
Adjusted Model[†]		0.346*		0.064*		**0.039***
Middle vs Low	0.33 (0.07, 1.65)	0.178	1.29 (0.49, 3.40)	0.601	0.26 (0.09, 0.74)	**0.012**
High vs Low	0.33 (0.07, 1.59)	0.166	0.31 (0.08, 1.17)	0.083	0.77 (0.22, 2.72)	0.689

*global p-value for chi-square test with 2 degrees of freedom; [†]adjusted for age, race, site, menopausal status, and BMI; TAT, total heart adipose tissue; CAC, coronary artery calcification; AC, thoracic aortic calcification; note: across TAT volume tertiles, the number of women with CAC present ranged from 1 to 8 for low volume, from 6 to 13 for middle volume, and from 10 to 39 for high volume; the number of women with AC present ranged from 3 to 12 for low volume, from 6 to 16 for middle volume, and from 8 to 38 for high volume.

Supplemental Table 6-2: Multivariable logistic regression for the associations between PVAT radiodensity tertiles and the presence of CAC ≥10 and AC ≥100 stratified by PVAT volume

CAC ≥10 Presence						
	Low PVAT Volume (n=174)		**Middle PVAT Volume (n=176)**		**High PVAT Volume (n=178)**	
PVAT Radiodensity Tertiles	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
Unadjusted Model		0.992*		0.154*		0.150*
Middle vs Low	0.93 (0.26, 3.23)	0.904	1.00 (0.25, 3.94)	0.995	1.78 (0.88, 3.61)	0.111
High vs Low	0.94 (0.27, 3.29)	0.926	2.45 (0.75, 8.05)	0.139	0.84 (0.39, 1.77)	0.641
Adjusted Model[†]		0.871*		0.163*		0.076*
Middle vs Low	1.05 (0.28, 3.88)	0.941	0.95 (0.23, 3.92)	0.941	2.44 (0.99, 5.99)	0.052
High vs Low	0.75 (0.20, 2.87)	0.679	2.50 (0.71, 8.74)	0.152	0.87 (0.35, 2.15)	0.758
AC ≥100 Presence						
	Low PVAT Volume (n=174)		**Middle PVAT Volume (n=176)**		**High PVAT Volume (n=178)**	
PVAT Radiodensity Tertiles	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
Unadjusted Model		0.090*		0.228*		0.156*
Middle vs Low	0.44 (0.14, 1.45)	0.177	1.08 (0.41, 2.83)	0.879	0.89 (0.43, 1.84)	0.762
High vs Low	1.50 (0.57, 3.92)	0.414	0.47 (0.16, 1.36)	0.164	0.46 (0.20, 1.03)	0.058
Adjusted Model[†]		0.194*		0.256*		0.197*
Middle vs Low	0.48 (0.14, 1.62)	0.237	0.91 (0.33, 2.48)	0.856	0.97 (0.40, 2.38)	0.943
High vs Low	1.52 (0.56, 4.10)	0.409	0.43 (0.14, 1.30)	0.135	0.44 (0.17, 1.14)	0.090

*global p-value for chi-square test with 2 degrees of freedom; [†]adjusted for age, race, site, menopausal status, and body mass index; PVAT, aortic perivascular adipose tissue; CAC, coronary artery calcification; AC, thoracic aortic calcification; note: across PVAT volume tertiles, the number of women with AC present ranged from 6 to 14 for low volume, from 7 to 12 for middle volume, and from 11 to 34 for high volume; the number of women with CAC present ranged from 5 to 6 for low volume, from 4 to 13 for middle volume, and from 16 to 33 for high volume.

Supplemental Table 6-3: Multivariable logistic regression for the associations between EAT radiodensity tertiles and the presence of CAC ≥10 and AC ≥100

CAC ≥10 Presence (n=488)			**AC ≥100 Presence** (n=486)		
EAT Radiodensity Tertiles	**OR (95% CI)**	**P-value**	**EAT Radiodensity Tertiles**	**OR (95% CI)**	**P-value**
Model 1		0.401*	**Model 1**		**0.008***
Middle vs Low	0.72 (0.41, 1.26)	0.248	Middle vs Low	0.55 (0.32, 0.94)	**0.030**
High vs Low	0.74 (0.45, 1.24)	0.258	High vs Low	0.47 (0.28, 0.78)	**0.004**
Model 2		0.434*	**Model 2**		**0.035***
Middle vs Low	0.71 (0.39, 1.29)	0.266	Middle vs Low	0.57 (0.32, 1.00)	0.050
High vs Low	0.70 (0.38, 1.30)	0.265	High vs Low	0.48 (0.26, 0.88)	**0.017**
Model 3		0.873*	**Model 3**		0.100*
Middle vs Low	0.84 (0.43, 1.63)	0.603	Middle vs Low	0.59 (0.33, 1.07)	0.083
High vs Low	0.92 (0.46, 1.85)	0.820	High vs Low	0.55 (0.29, 1.02)	0.057
Model 4		0.919*	**Model 4**		0.492*
Middle vs Low	0.87 (0.44, 1.73)	0.696	Middle vs Low	0.68 (0.36, 1.28)	0.234
High vs Low	0.97 (0.46, 2.04)	0.941	High vs Low	0.82 (0.45, 1.64)	0.571

*global p-value for chi-square test with 2 degrees of freedom; EAT, epicardial adipose tissue; CAC, coronary artery calcification; AC, aortic calcification; Model 1: unadjusted; Model 2: adjusted for age, race, site, menopausal status; Model 3: model 2 + body mass index; Model 4: model 3 + log-transformed triglycerides, smoking status, and systolic blood pressure.

Supplemental Table 6-4: Multivariable logistic regression for the associations between TAT radiodensity tertiles and the presence of CAC ≥10 and AC ≥100 stratified by race

CAC ≥10 Presence					
White Women			**Black Women**		
TAT Radiodensity Tertiles	**OR (95% CI)**	**P-value**	**TAT Radiodensity Tertiles**	**OR (95% CI)**	**P-value**
Unadjusted Model		**0.003***	**Unadjusted Model**		**0.010***
Middle vs Low	0.40 (0.20, 0.78)	**0.008**	Middle vs Low	0.18 (0.06, 0.55)	**0.002**
High vs Low	0.30 (0.12, 0.72)	**0.007**	High vs Low	0.39 (0.16, 0.95)	**0.038**
Adjusted Model†		0.234*	**Adjusted Model†**		0.279*
Middle vs Low	0.51 (0.23, 1.17)	0.112	Middle vs Low	0.34 (0.09, 1.31)	0.118
High vs Low	0.56 (0.19, 1.65)	0.290	High vs Low	0.70 (0.24, 2.11)	0.530
AC ≥100 Presence					
White Women			**Black Women**		
TAT Radiodensity Tertiles	**OR (95% CI)**	**P-value**	**TAT Radiodensity Tertiles**	**OR (95% CI)**	**P-value**
Unadjusted Model		**0.001***	**Unadjusted Model**		0.102*
Middle vs Low	0.47 (0.25, 0.86)	**0.015**	Middle vs Low	0.38 (0.13, 1.09)	0.071
High vs Low	0.23 (0.10, 0.56)	**0.001**	High vs Low	0.38 (0.15, 0.94)	**0.042**
Adjusted Model†		**0.040***	**Adjusted Model†**		0.788*
Middle vs Low	0.56 (0.27, 1.16)	0.120	Middle vs Low	0.65 (0.19, 2.26)	0.495
High vs Low	0.28 (0.10, 0.80)	**0.017**	High vs Low	0.76 (0.26, 2.27)	0.627

*global p-value for chi-square test with 2 degrees of freedom; †adjusted for age, race, site, menopausal status, body mass index, log-transformed triglycerides, current smoking status, and systolic blood pressure; TAT, total heart adipose tissue; CAC, coronary artery calcification; AC, thoracic aortic calcification; note: the number of women with CAC present ranged from 7 to 34 for Whites and from 7 to 26 for Blacks; the number of women with AC present ranged from 8 to 40 for Whites and from 10 to 20 for Blacks.

Supplemental Table 6-5: Multivariable logistic regression for the association between PVAT radiodensity tertiles and the presence of CAC ≥10 and AC ≥100 stratified by race

CAC ≥10 Presence					
White Women			**Black Women**		
PVAT Radiodensity Tertiles	**OR (95% CI)**	**P-value**	**PVAT Radiodensity Tertiles**	**OR (95% CI)**	**P-value**
Unadjusted Model		0.775*	**Unadjusted Model**		0.493*
Middle vs Low	0.80 (0.41, 1.55)	0.509	Middle vs Low	0.95 (0.43, 2.12)	0.905
High vs Low	0.84 (0.43, 1.63)	0.606	High vs Low	0.65 (0.29, 1.46)	0.297
Adjusted Model†		0.582*	**Adjusted Model**†		0.531*
Middle vs Low	1.36 (0.57, 3.26)	0.488	Middle vs Low	1.28 (0.43, 3.83)	0.658
High vs Low	1.52 (0.67, 3.46)	0.321	High vs Low	0.74 (0.24, 2.25)	0.593
AC ≥100 Presence					
White Women			**Black Women**		
PVAT Radiodensity Tertiles	**OR (95% CI)**	**P-value**	**PVAT Radiodensity Tertiles**	**OR (95% CI)**	**P-value**
Unadjusted Model		0.081*	**Unadjusted Model**		0.467*
Middle vs Low	0.62 (0.34, 1.16)	0.137	Middle vs Low	0.70 (0.31, 1.60)	0.404
High vs Low	0.49 (0.26, 0.95)	**0.035**	High vs Low	0.60 (0.27, 1.36)	0.224
Adjusted Model†		0.556*	**Adjusted Model**†		0.660*
Middle vs Low	0.68 (0.31, 1.48)	0.328	Middle vs Low	0.62 (0.22, 1.73)	0.363
High vs Low	0.69 (0.32, 1.49)	0.346	High vs Low	0.74 (0.27, 2.07)	0.569

*global p-value for chi-square test with 2 degrees of freedom; †adjusted for age, race, site, menopausal status, body mass index, log-transformed triglycerides, current smoking status, and systolic blood pressure; PVAT, aortic perivascular adipose tissue; CAC, coronary artery calcification; AC, thoracic aortic calcification; note: the number of women with AC present ranged from 16 to 36 for Whites and from 15 to 16 for Blacks; the number of women with CAC present ranged from 18 to 27 for Whites and from 15 to 20 for Blacks.

7.0 DISCUSSION

7.1 SUMMARY OF FINDINGS

This dissertation contains three manuscripts evaluating cardiovascular fat and cardiovascular risk factors in a community dwelling sample of White and Black midlife women. The first manuscript looked at determinants of the quantity of cardiovascular fat by assessing the associations between race, overall adiposity, and central adiposity with four separate cardiovascular fat depots. Interactions between race and adiposity measures in relation to cardiovascular fat quantities were assessed. The second manuscript assessed whether cardiovascular fat volumes were associated with serum adipokine/inflammatory marker levels and whether these associations differed by race. Further analyses looked at the associations between the cardiovascular fat quantities and CAC progression, independent of adipokine/inflammatory marker levels. The third manuscript extended our evaluation of cardiovascular fat as a risk factor for subclinical atherosclerosis by looking at a surrogate marker of fat quality as measured by computed tomography radiodensity. Associations between TAT and PVAT radiodensity values and the presence of CAC and AC were assessed.

In our first manuscript we found significant racial differences in the quantities of cardiovascular fat in which Black women had approximately 20% less EAT, 24% less PAT, 20% less TAT, and 13% less PVAT than White women. These differences were independent of age,

menopausal status, comorbidity, alcohol consumption, and physical activity. Further individual adjustments for BMI and SAT did not explain these differences. Interestingly, the reported differences between Black and White women in cardiovascular fat were somewhat attenuated when models were adjusted for VAT instead of BMI and SAT, suggesting a partial contribution of VAT in explaining the racial disparity in cardiovascular fat.

In addition, race modified the associations between some of the adiposity measures and cardiovascular fat volumes. White women had greater PAT than Black women for every 1 standard deviation increase in BMI; while Black women had greater EAT than White women for every 1 standard deviation increase in VAT. Although the analyses were cross-sectional and we were not able to assess temporality, these effect modifications by race indicate the possibility that the EAT depot in Black women may be more susceptible to VAT gain when compared with White women; whereas it is possible that the PAT and TAT depots in White women may be more susceptible to overall adiposity gain when compared with Black women.

Our second manuscript further evaluated the cardiovascular fat quantities and we found that EAT, PAT, and TAT volumes were significantly associated with serum leptin, LA ratio, and CRP levels, independent of cardiovascular risk factors, physical activity, alcohol consumption, and SAT. After adjusting for VAT, PAT volume remained significantly associated with leptin; however, all other associations between cardiovascular fat quantities and adipokine /inflammatory marker levels were attenuated. These findings emphasize the importance of evaluating cardiovascular fat depots separately. Race did not only modify associations between cardiovascular fat depots and adiposity measures, as we have reported in the first manuscript, but race also modified the associations between cardiovascular fat volumes and some of the adipokine and inflammatory marker levels. The positive associations between PAT and leptin

and LA ratio were more pronounced in White women than Black women; White women had 4% more leptin for each 20% increment increase in PAT, compared with Black women.

The third manuscript extends our assessment of cardiovascular fat by utilizing cardiovascular fat radiodensity as a surrogate marker of fat quality. We found that TAT radiodensity was associated with several CVD risk factors, with women in the lowest radiodensity tertile having a less favorable cardiovascular profile. Additionally, TAT radiodensity was associated with the presence of CAC; however, this association was not linear. Interestingly, women with mid-range TAT radiodensity values had a lower odds of CAC presence, independent of CVD risk factors and BMI, when compared to women with low radiodensity values. Although we anticipated finding an association between PVAT radiodensity and AC due to the close proximity of the fat to the aorta, no associations were found.

7.2 OVERALL DISCUSSION AND CONCLUSION

Cardiovascular fat has been shown in numerous studies to be a risk factor for CVD, independent of other measures of adiposity. This dissertation highlights unique aspects of cardiovascular fat that have yet to be investigated and is of considerable public health importance. With a better understanding of cardiovascular fat and cardiovascular risk we may be able to direct specific interventions designed to target vulnerable populations in hopes of reducing the risk of CVD. Midlife women are one such population due to the increased risk of CVD seen in postmenopausal women.[18] Previous analyses among the SWAN Cardiovascular Fat study participants have shown that late peri-/postmenopausal women have approximately 20% more PAT than pre-/early peri-menopausal women, which may correspond to an 11% increase in coronary events as reported in the Heinz Nixdorf study.[52,121]

Another vulnerable population includes Black women due to the increased risk of diabetes and CVD.[188,190] Among the Atherosclerosis Risk in Communities (ARIC) Study, with 9 years of follow-up, the risk of diabetes was almost 2.4 fold greater in Black women compared to White women and that approximately 48% could be due to modifiable factors.[191] Studies have shown that Black women tend to have higher BMI levels, but after accounting for BMI they carry less VAT and more SAT.[192,193] Since VAT is more strongly associated with cardiometabolic risk, the question naturally arises whether Black women experience the adverse effects of VAT at a lower threshold compared to White women; or does the extra SAT that Black women carry play more of a role in cardiometabolic risk.[223] These questions remain unanswered; however, this dissertation has shown that irrespective of their lower VAT, Black

women had more EAT (32% higher) than White women (25% higher) for each 1 standard deviation increase in VAT. Therefore, any change in VAT would have more detrimental effects on EAT in Black than in White women. It is possible that Black women are better able to buffer excess energy in SAT depots; however, once this fat becomes dysfunctional and begins to accumulate in the VAT depot, the rate of increase in EAT may be accelerated compared with White women. Although we are unable to assess this theory due to the cross-sectional design of our study, our results provide justification for further longitudinal analyses.

Our findings of Black women having significantly less cardiovascular fat volumes compared with White women, independent of adiposity measures were consistent with the results among men in the ERA-JUMP study.[15] Interestingly, the partial attenuation in the racial differences in cardiovascular fat that we found after adjusting for VAT was similar to the diminution found among the ERA-JUMP population of men.[15] In addition, the interactions reported between race and adiposity measures in our study were comparable to the effect modifications reported among men, with the magnitude of associations between BMI and cardiovascular fat volumes greater in Whites compared with Blacks.[15]

To date, most studies have investigated the EAT and TAT depots without looking at PAT as a separate depot. Literature suggests that EAT and PAT may differ in embryonic origin, adipocyte characteristics, and metabolic activity.[21,22] The results from this dissertation work support the theory that cardiovascular fat depots should be evaluated separately. It is well-recognized that adipose tissue is not simply an energy reserve, but a highly active metabolic endocrine organ which imposes differing cardiometabolic risk depending on the location in the body.[223] Our findings that only the PAT depot was significantly associated with leptin after adjusting for several potential confounders such as HOMA-IR, physical activity, cardiovascular

risk factors, and VAT or SAT, indicates the potential importance of this neglected cardiovascular fat depot. In addition, previous work in the SWAN Cardiovascular Fat study showed that a 4-year decline in estradiol was associated with higher PAT volumes, but was not related to EAT, TAT, or PVAT volumes, suggesting a potential role of sex hormones in controlling this depot. In fact, the median PAT volume (11.4 cm^3) among late peri-/postmenopausal women in our population (mean age, 52.6±2.7 years) was similar to the median PAT volume (12.4 cm^3) found among men (mean age, 45.3±2.8 years), and significantly more than PAT volume (8.4 cm^3) among pre-/early peri-menopausal women (mean age, 49.5±2.2 years).[15,52] These findings combined with our findings that White women had 14% more PAT for each 1 standard deviation increment change in BMI compared with Black women, suggests that the PAT depot should be further investigated to understand its importance among midlife women, especially among White women. It is intriguing to think that this may lend credence to the theory that when compared to Black women, White women are less able to buffer excess energy in SAT depots and they may begin to accumulate cardiovascular fat faster contributing to their higher volumes of cardiovascular fat. Although the importance of evaluating cardiovascular fat depots separately has already been suggested in literature, very little evidence is available to support this notion.[21,34] In fact, most studies have proposed assessing cardiovascular fat depots separately based on the importance of the EAT depot and PAT has been often overlooked.[21] Although all three cardiovascular fat depots are important, this dissertation provides evidence to spur additional research to evaluate the PAT depot as a cardiovascular risk factor.

Evidence has shown that the quality of fat is a risk factor for cardiometabolic risk; however, most studies evaluating fat quality are invasive.[39,40] Developing a noninvasive means of assessing fat quality, especially for cardiovascular fat depots, may provide valuable insight to

cardiovascular risk for midlife women. A few studies have used the radiodensity of fat as a surrogate marker of fat quality; however, the results are inconclusive. In general, fat quality, measured via radiodensity, has been shown to be related to CVD risk which indicates that it may provide insight to understanding the risks associated with cardiovascular fat. However, we still do not know how to interpret this measure and need more research to understand it. Our most intriguing finding was the non-linear effect of TAT radiodensity on the presence of CAC. Women with mid-range radiodensity values were the least likely to have CAC present. It has been hypothesized that high radiodensity may also represent fibrosis, which may convolute our understanding of radiodensity as a marker of fat quality.[45] Finding this non-linear effect backs the theory of fibrotic adipose tissue and provides support for future studies to evaluate fibrotic adipose tissue and to develop a more encompassing method of using radiodensity as a surrogate marker of fat quality. In addition, all studies to date have been cross-sectional and do not reflect the dynamic changes that may occur with this measure.

This dissertation research work should be viewed in the context of some limitations that include the cross-sectional nature which prevented us from assessing temporality. Our population only included Black and White midlife women which limits generalizability to other races/ethnicities, age groups, and men. Cardiovascular fat radiodensity consisted of one mean value for the entire fat depot and exactly what radiodensity measures remains unclear. In addition, due to the way our cardiovascular fat depots were measured, we did not have the radiodensity data for the PAT depot, which seems especially relevant to women at midlife. Adipokine and inflammatory marker levels were measured approximately 1 year after the cardiovascular fat measurements, ideally these measures would have been acquired at the same time point. Lastly, since our population consisted of healthy women with minimal CAC at

baseline, the 2.3 mean years of follow-up did not provide enough time for CAC to progress in this population. There is also the possibility that selection bias was introduced into these analyses since only 222 women were included from the 562 SWAN Heart women at baseline. This research has several strengths that include available data from the well-respected SWAN parent study. We had cardiovascular fat volumes for separate depots which provided us with the opportunity to assess the importance of each one individually. We had several adipokine and inflammatory marker levels available and high-quality measurements of cardiovascular fat depots, SAT, and VAT.

In conclusion, this research is of significant public health importance because it highlighted several important cardiovascular fat characteristics that include the following: 1) PAT may be an especially relevant cardiovascular fat depot for midlife White women; 2) cardiovascular fat radiodensity may be an indicator of cardiovascular risk in women at midlife; 3) among midlife women, Blacks had less cardiovascular fat compared to Whites; and 4) race modified associations between adiposity measures and cardiovascular fat volumes, as well as between cardiovascular fat volumes and serum leptin. Future studies should evaluate PAT as a separate fat depot and further explore its associations with cardiovascular risk. More research is necessary to understand fat radiodensity as a surrogate marker of fat quality and whether high radiodensity values are measuring fibrotic adipose tissue or factors such as macrophage accumulation or capillary density. Although many studies have found that cardiovascular fat depots are associated with cardiovascular risk, very limited information is available assessing how changes in cardiovascular fat influence cardiovascular risk and this should be a priority for future studies. Lastly, assessing whether there are racial differences in the manner in which

cardiovascular fat accumulates overtime may help to elucidate important areas of potential adiposity related CVD risk.

The clinical implications are three-fold. Firstly, cardiovascular fat depots are important cardiovascular risk factors for midlife women. This particular population experiences adipose tissue distribution changes with higher amounts of visceral and cardiovascular fat seen in postmenopausal women and these changes may account for some of the increased cardiovascular risk seen in postmenopausal women. The importance of the often unmeasured PAT depot may help us to better understand cardiovascular risk among this vulnerable population. Secondly, the cross-sectional racial differences in cardiovascular fat and in the associations between adiposity and cardiovascular fat may be an indication of important racial differences in the manner in which women accumulate fat. Since VAT is considered to be more strongly associated with cardiovascular risk, it is important to have a better understanding of why Black women have a stronger association between VAT and EAT compared to White women and whether this increases their risk of diabetes and CVD. Lastly, fat quality measures have been associated with cardiovascular risk factors; however, these measures are invasive which prevents large-scale evaluation. Developing a noninvasive method of assessing cardiovascular fat quality may shed light on the mechanisms in which these fat depots become dysfunctional and adversely affect local vasculature. We are in the very early stages of understanding radiodensity as surrogate marker of fat quality; however, our findings of the non-linear effect between TAT radiodensity and CAC suggests that we need a better understanding of radiodensity and that one mean value for an entire fat depot may not provide all of the information needed to evaluate fat quality.

APPENDIX: TABLES

Appendix-Table 1: Summary of selected studies evaluating the associations between coronary artery calcification and cardiovascular events and all-cause mortality

Study	Participants	CAC Scoring	Events	Covariates	Results
Jain et al.,[152] 2011	Sample Size 4965 Age (years) 62±10 Female 52% Follow-up (median years) 5.8	Continuous *log(CAC+1)*	All CHD: MI (definite /probable), resuscitated cardiac arrest, definite angina, probable angina (if followed by revascularization), and cardiac death Stroke: Fatal, and nonfatal, excluding transient ischemic attacks Heart Failure: definite or probable All CVD: All CHD + stroke + heart failure + other atherosclerotic death, and other CVD death	Model 1: Age, gender, ethnicity, BMI, SBP, total cholesterol, HDL, diabetes, smoking, hypertension, and lipid medication Model 2: model 1 + IMT, LV mass, and LV mass/volume ratio	*All CHD Events* HR (95% CI) Model 1: 2.4 (1.9, 2.8) Model 2: 2.3 (1.9, 2.8) *Stroke Events* HR (95% CI) Model 1: 1.1 (0.8, 1.4) Model 2: 1.3 (0.8, 1.4) *Heart Failure Events* HR (95% CI) Model 1: 1.4 (1.1, 1.8) Model 2: 1.4 (1.1, 1.7) *All CVD Events* HR (95% CI) Model 1: 1.7 (1.5, 2.0) Model 2: 1.7 (1.5, 1.9) *Note: HR represents hazard for 1 standard deviation increase*
Blaha et al.,[151] 2009	Sample Size 44,052 Age (years) 54±10 Female 46% Follow-up (mean years) 5.6	Categorized 0 (*ref*) 1-10 >10	All-Cause Mortality	Age, gender, hypertension, smoking, diabetes, hyperlipidemia, and family history of CHD	*All-Cause Mortality Events* CAC HR (95% CI) 1-10 2.2 (1.6, 3.0) >10 8.4 (6.8, 102.9)
Budoff et al.,[154] 2009 (JACC)	Sample Size 6809 Age (years) 62±10 Female 53% Follow-up (median years) 3.75	Categorized 0 (*ref*) 1-100 101-400 >400	Incident CHD: MI (definite/probable), resuscitated cardiac arrest, fatal CHD, definite angina, and probable angina (if followed by revascularization)	Unadjusted	*Incident CHD Events* CAC HR (95% CI) 1-100 6.1 (2.5, 14.7) 100-400 9.6 (5.0, 22.6) >400 9.9 (4.1, 24.3)

Appendix-Table 1: Continued

Study	Participants	CAC Scoring	Events	Covariates	Results
Budoff et al.,[153] 2009 (AHJ)	Sample Size 3923 Age (years) 58±9 Female 61% Follow-up (median years) 4.1	Categorized 0 (*ref*) 1-10 *Note: participants with a CAC score >10 were excluded*	Hard CHD: MI and cardiac death All CHD: Hard CHD + resuscitated cardiac arrest, definite angina, and probable angina (if followed by revascularization)	Age, gender, race, LDL hypertension, HDL, diabetes, smoking, cholesterol medications, internal carotid IMT	*Hard CHD Events* CAC HR (95% CI) 1-10 3.1 (1.1, 8.8) *All CHD Events* CAC HR (95% CI) 1-10 3.0 (1.4, 6.7)
Wong et al.,[140] 2009	Sample Size 2303 Age (years) 56±10 Female 38% Follow-up (mean years) 4.4	Categorized <10 (*ref*) 10-99 100-399 ≥400	Hard CHD: MI and cardiac death Total CHD: Hard CHD + late revascularizations Total CVD: Total CHD + stroke	Framingham risk score	*Hard CHD Events* CAC HR (95% CI) 10-99 2.4 (0.3, 17.3) 100-399 10.5 (2.1, 53.9) ≥400 12.0 (2.2, 64.5) *Total CHD Events* CAC HR (95% CI) 10-99 3.7 (1.03, 13.3) 100-399 11.9 (3.8, 37.0) ≥400 19.6 (6.3, 60.8) *Total CVD Events* CAC HR (95% CI) 10-99 2.8 (0.9, 8.3) 100-399 8.8 (3.4, 23.1) ≥400 13.1 (5.0, 34.2)
Budoff et al.,[58] 2007	Sample Size 25,253 Age (years) 56±11 Female 46% Follow-up (mean years) 6.8	Categorized 0 (*ref*) 1-10 11-100 101-399 400-699 700-999 ≥1000	All-Cause Mortality	Age, gender, hypertension, hyperlipidemia, diabetes, family history of premature coronary disease, smoking, and ethnicity	*All-Cause Mortality Events* CAC RR (95% CI) 1-10 1.5 (0.7, 3.1) 11-100 3.6 (2.1, 6.2) 101-399 3.8 (2.2, 6.7) 400-699 5.8 (3.0, 6.7) 700-999 6.5 (3.4, 12.4) ≥1000 9.4 (5.4, 16.3)
Arad et al., [155] 2005	Sample Size 4613 Age (years) 59±6 Female 35% Follow-up (mean years) 4.3	Categorized 0 (*ref*) 1-99 100-399 ≥400	All Coronary Disease: coronary death, nonfatal MI, coronary bypass surgery, and percutaneous coronary angioplasty	Unadjusted	*All Coronary Disease Events* CAC RR (95% CI) 1-99 1.9 (0.8, 4.2) 100-399 10.2 (5.0, 22.6) ≥400 26.2 (12.6, 53.7)

CAC, coronary artery calcium; ref, reference; CHD, coronary artery disease; MI, myocardial infarction; CVD, cardiovascular disease; BMI, body mass index; SBP, systolic blood pressure; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; HR, hazard ratio; IMT, carotid intimal-media thickness; LV, left ventricular; JACC, Journal of the American College of Cardiology; AHJ, American Heart Journal.

Appendix-Table 2: Summary of selected studies evaluating the associations between thoracic aortic calcification and cardiovascular events and all-cause mortality

Study	Participants	AC Scoring	Events	Covariates	Results
Kalsch et al.,[147] 2013	Sample Size 4040 Age (years) 59±8 Female 53% Follow-up (mean years) 8.0	Dichotomous 0 (*ref*) >0 Continuous Log(AC+1)	Myocardial Infarction: nonfatal acute MI and coronary death All-Cause Mortality	Model 1: Age, gender, BMI, SBP, LDL, HDL, diabetes, smoking, and cardiovascular medication Model 2: model 1 + log(CAC+1)	**AC >0 vs AC =0** *MI Events* HR (95% CI) Model 1: 1.4 (0.9, 2.1) Model 2: 1.1 (0.7, 1.7) _____ *All-Cause Mortality* HR (95% CI) Model 1: 1.0 (0.8, 1.3) Model 2: 0.9 (0.7, 1.2) _____ **Log(AC+1)** *MI Events* HR (95% CI) Model 1: 1.06 (1.00, 1.1) Model 2: 0.98 (0.9, 1.05) _____ *All-Cause Mortality* HR (95% CI) Model 1: 1.06 (1.01, 1.1) Model 2: 1.03 (0.98, 1.08) *Note: HR represents hazard for 1 log unit increase*
Allison et al.,[159] 2012	Sample Size 4544 Age (years) 57±11 Female 43% Follow-up (mean years) 7.8	Dichotomous 0 (*ref*) >0	CVD Mortality: CVD listed as underlying cause of death on Social Security Index Non-CVD Mortality: CVD not listed as underlying cause of death on Social Security Index Total Mortality: CVD mortality + non-CVD mortality	Age, gender, BMI, smoking, diabetes, hypertension, dyslipidemia, family history of CVD	*CVD Mortality Events* AC HR (95% CI) >0 3.0 (0.8, 10.9) _____ *Non-CVD Mortality Events* AC HR (95% CI) >0 2.0 (1.1, 3.6) _____ *Total Mortality Events* AC HR (95% CI) >0 2.1 (1.2, 3.5)

Appendix-Table 2: Continued

Study	Participants	AC Scoring	Events	Covariates	Results
Budoff et al.,[162] 2011	Sample Size 6,807 Age (years) 62±10 Female 53% Follow-up (mean years) 4.5	Dichotomous 0 (*ref*) >0	Hard CHD: MI and coronary heart disease related death All CHD: Hard CHD + resuscitated cardiac arrest, definite angina, and probable angina (if followed by revascularization)	Model 1: Age, race, BMI, hypertension, LDL, diabetes, smoking, family history of heart attack, and cholesterol-lowering medication Model 2: model 1 + CAC categories (0, 1-100, 101-400, and >400) *Note: Results stratified by gender due to significant interaction*	**WOMEN** *Hard CHD Events* HR (95% CI) Model 1: 2.4 (1.03, 5.6) Model 2: 1.7 (0.72, 4.2) *All CHD Events* HR (95% CI) Model 1: 3.0 (1.6, 5.8) Model 2: 2.2 (1.1, 4.2) **MEN** *Hard CHD Events* HR (95% CI) Model 1: 1.34 (0.8, 2.2) Model 2: 1.05 (0.6, 1.7) *All CHD Events* HR (95% CI) Model 1: 1.3 (0.9, 1.8) Model 2: 0.9 (0.6, 1.3)
Santos et al.,[160] 2010	Sample Size 8,401 Age (years) 53±10 Female 31% Follow-up (median years) 5.0	Dichotomous 0 (*ref*) >0	All-Cause Mortality	Model 1: Age, gender, hypertension, dyslipidemia, diabetes, smoking, and family history of premature CHD Model 2: model 1 + CAC presence *Note: Results stratified by gender in sub-analyses*	**OVERALL** *All-Cause Mortality Events* HR (95% CI) Model 1: 1.8 (1.2, 2.6) Model 2: 1.6 (1.1, 2.3) **WOMEN** *All-Cause Mortality Events* HR (95% CI) Model 1: 1.8 (0.8, 3.7) Model 2: 1.6 (0.8, 3.4) **MEN** *All-Cause Mortality Events* HR (95% CI) Model 1: 1.8 (1.1, 2.7) Model 2: 1.6 (1.02, 2.5)

Appendix-Table 2: Continued

Study	Participants	AC Scoring	Events	Covariates	Results
Wong et al.,[140] 2009	Sample Size 2,303 Age (years) 56±10 Female 38% Follow-up (mean years) 4.4	Categorized <10 (*ref*) 10-99 100-399 ≥400	Hard CHD: MI and cardiac death Total CHD: Hard CHD + late revascularizations Total CVD: Total CHD + stroke	Framingham risk score	*Hard CHD Events* AC — HR (95% CI) 10-99 — 3.8 (1.1, 12.6) 100-399 — 2.9 (0.7, 12.1) ≥400 — 2.1 (0.4, 10.8) *Total CHD Events* AC — HR (95% CI) 10-99 — 2.0 (0.9, 4.6) 100-399 — 3.0 (1.3, 6.9) ≥400 — 2.1 (0.8, 5.6) *Total CVD Events* AC — HR (95% CI) 10-99 — 1.5 (0.7, 3.5) 100-399 — 2.3 (1.04, 5.0) ≥400 — 1.9 (0.8, 4.6)
Eisen et al.,[161] 2008	Sample Size 361 Age (years) 62 Female 15% Follow-up (range years) 4.5-6.0 *Note: All participants have stable angina pectoris*	Dichotomous 0 (*ref*) >0	All Cardiovascular: cardiac death, acute MI, refractory angina, and stroke All-Cause: All cardiovascular + non-cardiac death, heart failure, and peripheral revascularization	Age, gender, BMI, hypertension, smoking, ACE inhibitors, calcium channel blockers, diuretics, and CABG history	*All CV Events* AC — HR (95% CI) >0 — 2.8 (1.5, 5.2) *All-Cause Events* AC — HR (95% CI) >0 — 4.6 (1.2, 18.3)

AC, thoracic aorta calcium; Ref, reference; CAC, coronary artery calcification; MI, myocardial infarction; BMI, body mass index; SBP, systolic blood pressure; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; CAC, coronary artery calcium; HR, hazard ratio; CVD, cardiovascular disease; CHD, coronary artery disease; ACE, angiotensin-converting enzyme; CABG, coronary artery bypass graft.

Appendix-Table 3: Race as a potential risk factor for coronary artery and thoracic aortic calcification

Study	Participants	Outcome	Covariates	Results
Janssen et al.,[173] 2012 (SWAN Heart)	Sample Size 336 Age (years) 51±3 Female 100% Race/Ethnicity 69% White 31% Black	CAC 0 (*ref*) 1-9 10-99 >99	Unadjusted	*Percentages of CAC* White (n=232) / Black (n=104) CAC 0: 61% / 45% CAC 1-9: 23% / 26% CAC 10-99: 13% / 28% CAC >99: 3% / 1% ***P<0.01***
Woodard et al.,[172] 2012 (SWAN Heart)	Sample Size 540 Age (years) 50 (48, 52)[†] Female 100% Race/Ethnicity 61% White 39% Black	AC 0 (*ref*) 1-100 >100 CAC 0 (*ref*) 1-10 >10	Unadjusted	**AC** *Percentages of AC* White (n=331) / Black (n=209) AC 0: 35% / 23% AC 1-100: 43% / 54% AC >100: 22% / 23% ***P<0.01*** **CAC** *Percentages of CAC* White (n=331) / Black (n=209) CAC 0: 59% / 42% CAC 1-10: 23% / 32% CAC >10: 18% / 26% ***P<0.001***
Budoff et al.,[153] 2009 (MESA)	Sample Size 3,923 Age (years) 58±6 Female 61% Race/Ethnicity 34% White 31% Black	CAC 0 (*ref*) 1-10	Unadjusted *Note: participants with a CAC score greater than 10 were excluded from analyses.*	*Percentages* CAC 0 (n=3415) / CAC 1-10 (n=508) White: 33% / 41% Black: 31% / 26% Hispanic: 24% / 21% Chinese: 12% / 12% ***P<0.0001***

Appendix-Table 3: Continued

Study	Participants	Outcome	Covariates	Results
Takasu et al.,[146] 2009 (MESA)	Sample Size 6,814 Age (years) 63±10 Female 51% Race/Ethnicity 38% White 28% Black	AC 0 (*ref*) >0 CAC 0 (*ref*) >0	Age, gender, former smoker, current smoker, BMI, hypertension, diabetes, family history of heart attack, LDL, HDL, lipid lowering medications, CRP, interleukin-6, fibrinogen, and factor VIII	**AC** *Prevalence Ratio of AC>0* RACE — PR (95% CI) White — 1 Black — 0.65 (0.59, 0.74)*** **CAC** *Prevalence Ratio of CAC>0* RACE — PR (95% CI) White — 1 Black — 0.76 (0.72, 0.80)***
Takasu et al.,[167] 2008 (MESA)	Sample Size 6,814 Age (years) 63±10 Female 51% Race/Ethnicity 38% White 28% Black	AC 0 (*ref*) >0	Age, gender, BMI, physical activity, family history of heart attack, diabetes, hypertension, smoking, alcohol, LDL, HDL, triglycerides, lipid lowering medications, and CRP	**WOMEN** *Relative Risk of AC >0* RACE — RR (95% CI) White — 1 Black — 0.37 (0.29, 0.48)* **MEN** *Relative Risk of AC >0* RACE — RR (95% CI) White — 1 Black — 0.41 (0.31, 0.54)*
Kronmal et al.,[163] 2007 (MESA)	Sample Size 5,756 Age (years) 62 Female 21% Race/Ethnicity 40% White 27% Black Follow-up 2.4 years	CAC Incidence *(n=2,948)* & Progression *(n=2,808)*	CAC Incidence: age, sex, follow-up time, BMI, SBP, diabetes, and family history of heart attack CAC Progression: age, sex, follow-up time, BMI, blood pressure and cholesterol medications, LDL, triglycerides, diabetes, family history of heart attack, and creatinine	**CAC Incidence** *Relative Risk of CAC >0* RACE — RR (95% CI) White — 1 Black — 0.79 (0.65, 0.98)* **CAC Progression** *Absolute Difference in CAC* RACE — % Difference (95% CI) White — 1 Black — -10.9 (-17.1, -4.8)***
Jain et al.,[168] 2004 (DHS)	Sample Size 1,289 Age (years) 52±6 Female 48% Race/Ethnicity 52% White 48% Black	CAC ≤ 10 (*ref*) >10	Age and gender *Note: investigators systematically oversampled Blacks in order to achieve approximately 50% in the final sample. Men <40 years old and women <45 years old were excluded.*	*Odds Ratio of CAC >10* RACE — OR (95% CI) Black — 1 White — 1.20 (0.94, 1.54)

Appendix-Table 3: Continued

Study	Participants	Outcome	Covariates	Results
Lee et al.,[169] 2003 (PACC)	Sample Size 888 Age (years) 42±2 Female 16% Race/Ethnicity 78% White 22% Black	CAC 0 (*ref*) >0	BMI, triglycerides, lipoprotein(a), SBP, HDL, left ventricular hypertrophy, ST-T abnormalities, former smoker, military rank, highest education level, hemoglobin A1C, and fibrinogen *Note: all participants were US Army active duty between 40-45 years old. Current active duty service members are healthier than the general population.*	*Odds Ratio of CAC>0* RACE OR (95% CI) White 1 Black 0.39 (0.20, 0.78)**
Khurana et al.,[224] 2003	Sample Size 861 Age (years) 63±8 Female 100% Race/Ethnicity 85% White 15% Black	CAC ≤10 (*ref*) >10	unadjusted	*Odds Ratio of CAC >10* RACE OR (95% CI) White 1 Black 0.83 (0.51, 1.35)
Budoff et al.,[170] 2002	Sample Size 782 Age (years) 57±11 Female 31% Race/Ethnicity 58% White 22% Hispanic 14% Black 6% Asian	CAC 0 (*ref*) >0	Age, gender, hypertension, diabetes, smoking, hypercholesterolemia, and family history of coronary disease *Note: all study participants had clinical indications of coronary artery disease.*	*Odds Ratio of CAC>0* RACE OR (95% CI) White 1 Black 0.30 (0.19, 0.48)*** Hispanic 0.45 (0.30, 0.67)*** Asian 0.50 (0.24, 1.00)

Appendix-Table 3: Continued

Study	Participants	Outcome	Covariates	Results
Newman et al.,[54] 2002 (CHS)	Sample Size 614 Age (years) 80±4 Female 60% Race/Ethnicity 77% White 23% Black	CAC ***Women*** 0-30 (ref) 31-200 201-660 >660 ***Men*** 0-166 (ref) 167-625 626-1433 >1433	Age, presence of clinical CVD, SBP, DBP, BMI, diabetes, HDL, LDL, total cholesterol, triglycerides, ever smoked, pack-years of smoking, CRP, and fibrinogen	**WOMEN** *Odds Ratio of CAC>660* RACE OR (95% CI) White 1 Black 0.71 (0.44, 1.14) **MEN** *Odds Ratio of CAC>1443* RACE OR (95% CI) White 1 Black 0.19 (0.10, 0.35)***
Bild et al.,[171] 2001 (CARDIA)	Sample Size 443 Age (years) 35±4 Female 48% Race/Ethnicity 45% White 55% Black	CAC 0 (*ref*) >0	Age, gender, education, BMI, SBP, LDL, triglycerides, and fasting insulin	*Odds Ratio of CAC>0* RACE OR (95% CI) White 1 Black 0.87 (0.44, 1.73)

*p<0.05, **p<0.01, ***p<0.001; †Median (IQR); SWAN Heart, Study of Women's Health Across the Nation Heart Ancillary Study; CAC, coronary artery calcium; AC, thoracic aorta calcium; ref, reference; MESA, Multi-Ethnic Study of Atherosclerosis; DHS, Dallas Heart Study; PR, prevalence ratio; RR, relative risk; BMI, body mass index; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; CRP, C-reactive protein; PACC, Prospective Army Coronary Calcium project; CHS, Cardiovascular Health Study; SBP, systolic blood pressure; DBP, diastolic blood pressure; CVD, cardiovascular disease; OR, odds ratio; CARDIA, Coronary Artery Risk Development in Young Adults study.

Appendix-Table 4: Summary of selected studies evaluating cardiovascular fat and atherosclerotic calcification

Study	Participants	CV Fat	Arterial Calcium	Covariates	Results
Mahabadi, et al.,[35] 2014	Sample Size 3,367 Age (years) 59±8 Female 53% Race N/L BMI (kg/m^2) 28±4	Fat Depot EAT *(Continuous)* Measurement Volume (cm^3) HU range -195 to -45	CAC Progression (Agatston) *(Continuous)* Follow-up 5.1±0.3 years	Age, gender, BMI, SBP, DBP, BP and cholesterol medication, LDL, HDL, diabetes, triglycerides, smoking status	% Progression in log(CAC+1) for each 10cm^3 EAT increase *Linear Regression* 6.1%*
Wassel, et al.,[34] 2013	Sample Size 598 Age (years) 68±7 Female 76% Race 57% White 20% Filipina 23% Black BMI (kg/m^2) 27±4	Fat Depot EAT PAT *(Continuous)* Measurement Volume (cm^3) HU range -190 to -30	CAC Progression (Agatston) *(Continuous)* Follow-up 4.0 years	Age, gender, BMI, VAT, race, diabetes, lipids, smoking status, exercise, hypertension, adipocytokines	CAC Progression FAT OR (95% CI) EAT 1.09 (0.77, 1.55) PAT 1.70 (0.76, 3.79)
Shields, et al.,[83] 2013 (HEARTS)	Sample Size 311 Age (years) 50±10 Female 100% Race 89% White BMI (kg/m^2) 27	Fat Depot PVAT *(Continuous)* Measurement Volume (cm^3) HU range -190 to -30	AC 0 (*ref*) >0 (Agatston)	Waist-to-hip ratio, postmenopausal status, smoking, SBP, HOMA-IR, cholesterol ratio, homocysteine levels, CRP, fibrinogen, sICAM, PAI-1, eSelectin	*Odds Ratio of AC >0* Group OR (95% CI) SLE 4.52 (1.3, 15.0)* Controls 4.7 (1.8, 12.0)**
Bettencourt et al.,[14] 2012	Sample Size 215 Age (years) 58±11 Female 39% Race N/L BMI (kg/m^2) 28±4	Fat Depot EAT *(Continuous)* Measurement Volume (ml) HU range -150 to -50	CAC (Agatston) *(Continuous)*	Age, gender, visceral abdominal fat, waist circumference, obesity, diabetes, HbA1c, DBP, modified Diamond-Forrester estimations	Increase of CAC (%) by additional 10ml EAT *Poisson Regression* 3.7*** *Note: results are from cross-sectional analyses*

Appendix-Table 4: Continued

Study	Participants	CF Depot	Arterial Calcium	Covariates	Results
Huang et al.,[10] 2012 (KEEPS)	Sample Size 650 Age (years) 53±3 Female 100% Race 74% White BMI (kg/m^2) 26±0.3 *Note: recently menopausal women*	Fat Depot EAT & TAT (*Quartiles*) Measurement Volume (cm^3) HU range -190 to -30	CAC 0 (*ref*) >0 (Agatston)	Model 1: age, race/ethnicity, education level, smoking status, alcohol intake, physical activity level, and study center, BMI, and WC	**EAT** *Odds Ratio of CAC >0* EAT (cm^3) — OR (95% CI) <28.2 — 1.0 28.2-38.4 — 0.7 (0.3, 1.4) 38.5-49.7 — 1.5 (0.8, 2.8) >49.7 — 1.8 (0.9, 3.4) ***p for linear trend 0.020*** **TAT** *Odds Ratio of CAC >0* TAT (cm^3) — OR (95% CI) <32.29 — 1.0 32.29-44.70 — 0.7 (0.3, 1.4) 44.71-58.69 — 1.5 (0.7, 2.9) >58.69 — 1.8 (0.9, 3.3) ***p for linear trend 0.027***
Yerramasu et al.,[36] 2012	Sample Size 333 Age (years) 54* Female 38% Race 21% White 28% Black 51% Asian BMI (kg/m^2) 29±5	Fat Depot EAT (*Continuous*) Measurement Volume (cm^3) HU range -190 to -30	CAC (Agatston) 0 (*ref*) >0 Follow-up 2.7±0.3 years	Age, gender, race, waist to hip ratio, SBP, osteoprotegerin *Note: all participants had type II diabetes mellitus*	*Odds Ratio of CAC >0* OR (95% CI) 1.13 (1.04, 1.22)* *Note: Ratio given for a 10cm^3 increase in EAT volume* *CAC Progression* OR (95% CI) 1.23 (1.05, 1.19)***
Guaraldi et al.,[225] 2011	Sample Size 876 Age (years) 47±8 Female 32% Race N/L BMI (kg/m^2) 24±4	Fat Depot EAT (*Continuous*) Measurement Volume (cm^3) HU range -190 to -30	CAC (Agatston) ≤100 (*ref*) >100	Age, gender, and diabetes *Note: all participants are HIV+ and taking ART*	*Odds Ratio of CAC >100* OR (95% CI) 1.10 (1.02, 1.19)* *Note: Ratio given for a 10cm^3 increase in EAT volume*

Appendix-Table 4: Continued

Study	Participants	CF Depots	Arterial Calcium	Covariates	Results
Ahmadi et al.,[182] 2010	Sample Size 111 Age (years) 60±10 Female 42% Race N/L BMI (kg/m^2) 30±4	Fat Depot EAT, PAT, & TAT (*Continuous*) Measurement Volume (cm^3) HU range -190 to -30	CAC (Agatston) 0 (*ref*) ≥100	Age, gender, diabetes, hyper-cholesterolemia, hypertension, family history of CHD, smoking, and BMI	*Odds Ratio of CAC ≥100* FAT OR (95% CI) EAT 3.32 (1.95, 5.62)*** PAT 2.72 (1.64, 3.94)*** TAT 3.06 (1.87, 5.03)*** *Note: Ratio given for a 10cm^3 increase in EAT volume*
Lehman et al.,[82] 2010 Framingham Heart Study	Sample Size 1,067 Age (years) 59±9 Female 56% Race N/L BMI (kg/m^2) 28±5	Fat Depot PVAT (*Continuous*) Measurement Volume (cm^3) HU range -195 to -45	CAC (Agatston) 0 (*ref*) >0 AC (Agatston) 0 (*ref*) >0	Model 1: age, gender, SBP, hypertension treatment, diabetes, total/HDL cholesterol, lipid treatment, smoking, alcohol, menopausal status, HRT Model 2: model 1 + VAT	**AC** *Odds Ratio of AC >0* OR (95% CI) Model 1: 0.89 (0.73, 1.08) Model 2: 1.16 (0.88, 1.51) **CAC** *Odds Ratio of CAC >0* OR (95% CI) Model 1: 1.11 (0.90, 1.36) Model 2: 1.47 (1.09, 1.98)* *Note: Ratio given for a 1-SD increase in PVAT*
Mahabadi et al.,[226] 2010	Sample Size 78 Age (years) 61±12 Female 32% Race N/L BMI (kg/m^2) 26±4	Fat Depot EAT (*Continuous*) Measurement Volume (cm^3) HU range -195 to -45	CAC (Agatston) 0 (*ref*) >0	Age and gender	*Odds Ratio of CAC >0* OR (95% CI) 2.44 (1.78, 3.32)*** *Note: Ratio given per each doubling of EAT volume*
Ding et al.,[227] 2008 (MESA)	Sample Size 159 Age (years) 65±5 Female 50% Race 50% White 50% Black BMI (kg/m^2) 29±4	Fat Depot TAT (*Continuous*) Measurement Volume (cm^3) HU range -190 to -30	CAC (Agatston) 0 (*ref*) >0	Age, gender, race, height, smoking, alcohol, SBP, CRP, statins use, hypertension medication, lipid-lowering medication, diabetes, hypertension medication, total and HDL cholesterol	*Odds Ratio of CAC >0* OR (95% CI) 2.10 (1.28, 3.45)** *Note: Ratio given for a 1-SD increase in TAT volume*

Appendix-Table 4: Continued

Study	Participants	CF Depots	Arterial Calcium	Covariates	Results
Rosito et al.,[88] 2008 (Framingham Heart Study)	Sample Size 1,155 Age (years) 63±9 Female 55% Race N/L BMI (kg/m^2) 28±5	Fat Depot EAT & TAT (*Continuous*) Measurement Volume (cm^3) HU range -195 to -45	CAC (Agatston) 0 (*ref*) >0	Model 1: Age, gender, SBP, hypertension medication, diabetes, total/HDL cholesterol, lipid treatment, smoking, alcohol, menopausal status, and HRT Model 2: model 1 + VAT	**EAT** *Odds Ratio of CAC >0* OR (95% CI) Model 1: 1.13 (0.97, 1.31) Model 2: 1.21 (1.01, 1.46)* **TAT** *Odds Ratio of CAC >0* OR (95% CI) Model 1: 1.10 (0.93, 1.30) Model 2: 1.23 (0.97, 1.57)

*p<0.05; **p<0.01; ***p<0.001; N/L, not listed; HbA1c, Hemoglobin A1c; DBP, diastolic blood pressure; CAC, coronary artery calcium; HU, Hounsfield units; KEEPS, Kronos Early Estrogen Prevention Study; TAT, total heart adipose tissue; BMI, body mass index; WC, waist circumference; SD, standard deviation; MESA, Multi-Ethnic Study of Atherosclerosis; EAT, epicardial adipose tissue; PAT, pericardial adipose tissue; ART, antiretroviral therapy; HIV+, human immunodeficiency virus positive; CHD, coronary heart disease; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; SBP, systolic blood pressure; HRT, hormone replacement therapy; VAT, visceral adipose tissue; CRP, C-reactive protein; HOMA-IR, homeostatic model assessment of insulin resistance; sICAM, soluble intercellular adhesion molecule; Note: the terminology to define cardiovascular fats varies between studies and to prevent confusion, EAT is defined as the fat within the pericardial sac, PAT is defined as the fat outside the pericardial sac, TAT is defined the combination of EAT and PAT, and PVAT is defined as peri-aortic fat, regardless of the terminology used in each study.

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