Periaortic Adipose Tissue Relationship with Arterial Stiffness in Midlife Men

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Abstract

Aims: Cardiovascular disease (CVD) remains a major health concern and leading cause of death in the United States. Arterial stiffness is part of the aging process and reflects altered vascular function and may eventually lead to adverse cardiovascular events. Carotid-femoral pulse wave velocity (cfPWV) is the gold standard of measuring arterial stiffness and a predictor of CVD. Periaortic adipose tissue (aPVAT) is an emerging CVD risk marker. Because of its anatomy, aPVAT may directly affect vascular function. We hypothesized that 1) aPVAT surrounding the descending thoracic aorta would be associated with cfPWV in a biracial cohort of middle-aged men and 2) the association between aPVAT and cfPWV will be stronger in white men compared to African-American men

Methods: Participants (n=324; whites=251, African-Americans=73) were 40-49 years old men from the ERA-JUMP (Electron-Beam Tomography Risk Factor Assessment Among Japanese and U.S. Men in the Post-World War II Birth Cohort) study and were free of clinical CVD, type-1-diabetes, or other severe disease at the time of recruitment. cfPWV was measured using an automated waveform analyzer and aPVAT was quantified using electron beam computed tomography scans of the aorta with standard attenuation values for adipose tissue (-190 to -30 HU).

Results: Whites were less likely to be current smokers and hypertensive (both $p<0.05$), but had more aPVAT ($p=0.011$) and visceral adipose tissue (VAT;$p<0.0001$) compared with African American men. There was no difference in cfPWV by race. aPVAT was positively associated with

cfPWV independent of traditional CVD risk factors (p=0.005). The association between aPVAT and cfPWV persisted after adjusting for adiposity measurements, such as VAT (p=0.054), waist circumference (p=0.038), or BMI (p=0.052), CRP, and hypertensive, lipid, and diabetes medications. Race did not moderate the relationship between aPVAT and cfPWV (p=0.418).

Conclusion: Middle-aged men with high periaortic fat volume are more likely to have stiffer arteries independent of traditional risk factors, adiposity measurements, a non-specific marker inflammation, and medications for cardiometabolic conditions. The public health significance of this research is based on its contribution to a deeper understanding of the role of aPVAT as an ectopic fat with potential effects on vascular function beyond risk factors and overall adiposity in middle-aged men.

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Preface

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1.0 Introduction

1.1 Cardiovascular Disease

1.1.1 Epidemiology

Cardiovascular disease (CVD), a major public health concern and a leading cause of death in the United States, is responsible for one of every three deaths (1). This is more deaths than all forms of cancer and chronic lower respiratory disease combined. According to the American Heart Association (AHA), CVD accounted for more than 17.6 million deaths per year in 2016 and the number is expected to grow to more than 23.6 million by 2030 (1). Although CVD related mortality has been declining since the 1970's (2) (for example, from 2006 to 2016 the annual death rate attributable to coronary heart disease (CHD) declined 31.8%), the burden and risk factors remain high (1). AHA stated that between 2014 and 2015, cost of CVD and stroke were $351.2 billion, both direct and indirect, and this is projected to increase to $749 billion in 2035 (1). CVD is considered the most costly disease and its direct cost is estimated to be more than cancer (3).

Moreover, the decline in CVD mortality has not been experienced equally among certain demographic groups. For example, declining heart disease death rates were slower in African-Americans than whites (2). Around 2010, the decline leveled for both races, with consistently higher rates among African-Americans compared to whites (2). There are several factors contributing to this race difference. First, a recent surveillance report suggests that African-Americans did not benefit equally from implementation of prevention programs and treatment of heart disease compared to whites (2). Emerging evidence suggests that race disparities in CVD are

due to a differences in prevalence of certain traditional CVD risk factors: obesity, high blood pressure, and history of diabetes (4,5). Other pathways beyond traditional risk factors including socioeconomic, cultural and novel biological risk factors are likely to play a role and merit further investigation (6).

It is well established that the prevalence of CVD is greater in men than in women and sex hormones are likely to be involved in this difference (7). In addition to sex, the main cardiovascular risk factors include age, dyslipidemia, high blood pressure, obesity, history of diabetes, smoking, physical inactivity, and poor nutrition (5). CVD also has a well-established pathway and surrogate biomarkers linked to inflammation including C-reactive protein (CRP), interleukin-6 (IL-6), fibrinogen, and tumor necrosis factor alpha (TNFα) (8).

1.1.2 Pathophysiology

CVD refers to several heterogeneous disorders affecting the heart and vasculature, the majority of which are caused by injury to and thickening and stiffening of the arterial walls. Coronary heart disease is caused by disruption of blood supply to the heart muscle (9). Stroke is caused by disruption of blood supply to the brain (9). These two have the highest mortality in developing countries (1). Other types include heart failure, refers to heart pumping insufficient blood due to cardiac structural and functional abnormalities, and peripheral arterial disease, refers to insufficient supply to arteries and vessels outside of the heart, including the lower extremities (9).

Atherosclerosis is the underlying cause of most common cause of CVD and is considered an inflammation-mediated disease process. Once considered to be a simple lipid-accumulation disease within the arterial wall, atherosclerosis is driven by a progressive inflammatory cascade in

response to injury to the arterial wall which worsens with risk factors, such as hypertension or obesity. The formation of lesions in atherosclerosis, called plaques, which reside in the arterial walls, are the hallmark of atherosclerotic CVD. These plaques can rupture or erode and ultimately cause sudden local or downstream thrombotic occlusion leading to catastrophic CVD events, such as myocardial infarction and downstream heart failure (10). The initiation of atherosclerosis development is accelerated by recruitment of inflammatory monocytes into the vessel wall. This increase activates the adherence of monocytes to vascular cell adhesion molecule-1 (VCAM-1) (10). Then chemokines induce monocytes to enter the arterial intima, where they mature into macrophages (10). Uptake of oxidized low-density lipoproteins (LDL) by macrophages forms foam cells, which amplify the inflammatory process by cytokines (10). These cytokines can enhance oxidation of LDL, which leads to secretion of inflammatory mediators to express more VCAM-1 and the vicious cycle begins again (10).

Arterial stiffness is thought to be part of the aging process that alters vascular function, especially in the large elastic arteries such as the aorta resulting in elevated systolic blood pressure. These changes are due to fragmentation and degeneration of elastin, increased amount of collagen, and extracellular matrix calcification which result in arterial structural changes and subsequent reduction in vascular distensibility (11). This degeneration of the arterial wall occurs at a different time and rate for each individual, which is determined and exacerbated by risk factors, such as high blood pressure and obesity (12). Because of the increased central aortic pressure, due to the reflected pressure wave returning earlier in the cardiac cycle, arterial stiffening may result in greater left ventricular afterload and isolated systolic hypertension. These consequences of arterial stiffness may eventually lead to progressive left ventricular hypertrophy, which is thickening of the walls of the heart because of the increased workload, decreased coronary perfusion, and/or

microvascular damage (11). It may eventually result in a cardiovascular event, such as a stroke or heart failure.

Arterial stiffening is the degeneration of the extracellular matrix and the fatigue of the elastin, both of which are different from atherosclerosis (13). Often both these processes, arterial stiffening and atherosclerosis, coexist in the same vascular regions, sharing mutual risk factors, and are a large part of the vascular aging process (13). The relationship between arterial stiffness and atherosclerosis is not entirely established due to the fact that it is complex and involves a number of different mechanisms, such as mechanical and metabolic (13). Both pathological processes involve chronic inflammation. For example, C-reactive protein (CRP), known as a non-specific biomarker of vascular inflammation, has a positive relationship with both atherosclerosis and arterial stiffness (14,15). Over the years, many studies have shown arterial stiffness is an independent predictor of cardiovascular morbidity and mortality as it is correlated with vascular calcification and peripheral pressure (16). Given that arterial stiffness offers good insight predicting CVD, and can be measured early in the disease process before events, factors that influence arterial stiffness should be explored.

Pulse wave velocity (PWV) is a well-known marker of arterial stiffness. It is a measurement of the ratio of the distance between two arterial sites divided by the time required for the pressure wave to travel to those arterial sites (13). There are many arterial sites that PWV can be measured, such as heart-femoral or brachial-ankle. A higher PWV indicates stiffer arteries. Carotid-femoral PWV (cfPWV) represents the gold standard for arterial stiffness measurement and is a well-known predictor of adverse cardiovascular events (17). For this reason, cfPWV is a primary outcome in this study.

1.2 Obesity and Cardiovascular Disease

Obesity is a major predisposing risk factor for CVD (4). The World Health Organization (WHO) has reported that obesity has tripled since 1975 (18). In the United States, nearly 78 million adults are dealing with the health effects of obesity (19). The age-adjusted prevalence of obesity is highest among Hispanic men (47.0%) and African-American men (46.8%), followed by white men (37.9%) and Asian men (12.7%), during 2015 to 2016 (20). This pattern is similar among women (20). As one of the main risk factors for CVD, the global increase in obesity will lead to a further increase of CVD in the next decade (21). An increase in the prevalence of obesity is also a possible reason for the leveling of the heart disease death rate around 2010, undermining the gains attributed to the decrease of other risk factors such as hypertension and smoking (10).

There are numerous adverse effects of obesity on cardiovascular health. Obesity may act directly by affecting cardiovascular structure and function and/or indirectly by worsening traditional risk factors. Independent of arterial pressure and age, obesity increases the risk of left ventricular hypertrophy and the abnormality of concentric remodeling (22). This structural abnormality increases the risk of heart failure and has adverse effects on diastolic and systolic function (22,23). For an indirect effect example, obesity pathologically affects blood pressure and can lead to CVD, such as hypertension and heart failure, and related comorbidities including type 2 diabetes mellitus and sleep apnea (22,24,25). In addition, it adversely affects triglycerides and prothrombotic factors (26). Environmental factors, such as the excessive consumption of fatty foods and a sedentary lifestyle, can lead to obesity and can also significantly contribute to atherosclerosis, independently of obesity, ultimately causing CVD (27). However, not all obese individuals will experience CVD. Studies are investigating body composition and fat distribution as suspects in the pathogenesis of obesity-induced CVD beyond overall adiposity.

Obesity also might exert adverse effects on the vascular systems by augmenting arterial stiffness. One of the first studies to demonstrate the relationship between obesity and arterial stiffness concluded that obese patients have stiffer arteries compared to non-obese patients independent of age, sex, blood pressure, fasting blood glucose level, cholesterolemia, and triglyceridemia (28). It has been principally believed that arterial stiffness is determined by age and blood pressure but later demonstrated that contribution of other risk factors including obesity is also important to arterial stiffness (13,29). Over the years, studies have shown a significant association between PWV and waist circumference (WC), indicating that the location of fat plays a role in the pathogenesis of arterial stiffness (30,31). One theory is that increased adiposity contributes to low-grade inflammation and increased levels of immune system cells will migrate into the arterial wall, leading to stiffening of the artery (32). The association between adiposity and arterial stiffness starts out during the early years, which means this obesity-induced vascular effect is possibly acute and long-term, and potentially reversible (33).

1.2.1. Adipose Tissue Distribution

The increase in the prevalence of obesity is changing the etiology of CVD, which many view as a consequence of adipose tissue dysfunction (34). Excessive caloric intake leads to changes in the quantity, structure, and cellular composition of adipose tissue (34). Decades ago, adipose tissue was regarded as an inert fat storage organ, then it became apparent that it secretes hundreds of biologically active peptides, such as cytokines and adiponectin, which can modulate inflammatory and metabolic processes, resulting in a chronic vascular inflammation state, either by a paracrine or endocrine (systemic) effect (35). Often obese patients have increased oxidized lipids, which leads to accelerated cytokine production and results in atherosclerosis (10). Pro-

inflammatory cytokines, such as interleukins, may also play a role in the development of arterial stiffness in obese individuals. (13,29).

In much of the population-based research, the extent of obesity is categorized using body mass index (BMI), which is weight in kilograms divided by height in meters squared. The advantage of this measurement is that the information needed to calculate it is easy to collect, and it is a common tool used in both research and clinical based settings. Obesity is a concept that refers to excessive fatness and commonly defined as a BMI $\geq$ 30 kg/m^2. Even though multiple health organizations recommend using BMI to identify obesity in individuals, the optimal approach to measure adiposity at the population level remains uncertain. The importance of regional adiposity being a better predictor of CVD than BMI has been debated (38).

Some studies have focused on the location and type of adipose tissue as a predictor of cardiovascular risk because overall adiposity alone does not account for the full spectrum of adipose biology (36,37). Obesity defined using BMI results in substantial misclassification of individuals into weight classifications because it does not separate fat from muscle. This can result in overestimating BMI since muscle is more dense than fat (38). When the percent of body fat is used to define obesity, instead of BMI, the gap in obesity between African American and white women is cut in half (38). There is increasing evidence that the location of adipose tissue is an important factor in cardiometabolic risk for an individual because the location of the fat deposit may dictate physiology (36,39). While many studies have agreed that BMI is a poor measurement for adiposity, there is no agreement on which measure is the best and most accurate (37,40). Adipose tissue conveys varying cardiovascular risk depending on the location in the body and its anti- and pro- atherogenic cytokines may link obesity to CVD by systemic and local inflammatory effects (41,42).

Locations of fat deposits that have been well studied in the epidemiology literature include subcutaneous adipose tissue (SAT), which is beneath the skin, and visceral adipose tissue (VAT), which surrounds the internal organs. Visceral fat depots in important organs, such as kidneys and the heart, have been associated with organ dysfunction (43). As opposed to SAT, VAT has shown to be the driving force behind many adverse cardiovascular risk factors and mediators, including obesity, hypertension, and insulin resistance. For example, the Framingham Heart Study (FHS) found that VAT has a stronger correlation with cardiovascular risk factors (such as WC, HDL etc.) than BMI (44). The FHS also reported evidence of VAT related to increased systolic blood pressure, fasting plasma glucose, and diabetes mellitus (45). Premenopausal women have significantly more SAT whilst men have more VAT, highlighting well-known sex differences in body fat distribution (46). Fat distribution is also influenced by ethnic background. In the Multicultural Community Health Assessment Trial (M-CHAT), the Chinese and South Asian populations had significantly more VAT compared to Europeans (46). Another multi-ethnic study found that both white and Hispanic men had greater VAT than African-American men after controlling for total body fat; similar results were observed in women (47). The Health ABC study confirmed that increased aortic stiffness was shown to be associated with VAT, better than BMI (48). These studies suggest that location of adipose tissue may be a better indicator of CVD risk than overall adiposity.

More recently, cardiovascular fat, that is adipose tissue surrounding the heart (epicardial and paracardial) and blood vessels (perivascular), has been extensively studied (49). For example, in the SWAN cardiovascular fat ancillary study, healthy postmenopausal women had more paracardial adipose tissue compared to premenopausal women. This greater amount of paracardial adipose tissue had a significant association with a greater coronary artery calcification (CAC),

which is a strong indicator for future cardiovascular risk (50). In addition, FHS concluded that epicardial fat is associated with vascular calcification and increased inflammatory markers among those free of CVD. This suggests that fat proximal to the coronary arteries may contribute to deleterious local effects on the vasculature (51).

1.2.2. Periaortic Adipose Tissue and Vascular Function

Perivascular adipose tissue (PVAT), a novel ectopic fat depot, is located outside of the blood vessel wall and connected with the adventitial layer (52). It is known to be an active contributor to vascular function, associated with inflammation and endothelial dysfunction (39,52). PVAT loses its normal vascular protective effects in obesity because of abnormal production of adipokines, such as tumor necrosis factor-α (53). In a small study of obese patients, removing PVAT from small arteries restored endothelial function (54). PVAT likely enhances the risk of developing CVD in individuals with obesity through altering vascular function.

Periaortic adipose tissue (aPVAT) is a type of PVAT that is in contiguity with the aorta and a recent emerging novel risk marker for CVD (52). Because of its anatomy, aPVAT inflammation may have a direct effect on vascular function, potentially leading to development of atherosclerosis. For example, aPVAT adipocytes release soluble protein that stimulate growth of the smooth muscle cell, which is known to aggravate vascular disease (55). Furthermore, aPVAT, in the high-fat-diet obesity-induced C57BL/6 mice, promoted proinflammatory chemokines and other cytokines, which lead to phenotypic alterations in the vascular wall (56). Adipokines, such as leptin and chemerin, are known to be associated with chronic inflammation (57,58) These adipokines expression in aPVAT was highly correlated with severity of aortic atherosclerosis (59). This confirms the relationship between aPVAT and vascular disease may exist. Recently, evidence from animal and human studies has implicated aPVAT in arterial stiffness. An animal study

demonstrated that superoxide signaling within aPVAT contributes to augmented arterial stiffness accompanied by greater production of inflammatory proteins (Table 1) (60). Correspondingly, another animal study showed that aPVAT presence promoted arterial stiffness in lipoprotein-receptor deficient mice with increased circulating cholesterol and triglyceride concentration (61). These animal model studies demonstrated potential effects of aPVAT on vasculature and should be studied extensively.

Population-based cohort studies looked at the relationship between aPVAT and CVD related risk factors and vascular outcomes, although they are mainly limited to the FHS. According to the FHS, peri-aortic fat volumes were associated with cardiovascular risk factors, such as serum cholesterol, blood pressure, and fasting glucose when adjusted for BMI and waist circumference (WC) (Table 1) (62). In agreement with the above observations, community-based FHS confirmed that aPVAT is associated with multiple measures of vascular function, such as arterial pressure, cfPWV, the augmentation index, and aortic dimensions, even after adjusting for BMI (63,64). Also extending findings in the FHS, another FHS paper showed that aPVAT is associated with the low ankle-brachial index, a marker of peripheral arterial disease, and aortic calcification (65–67). These findings establish aPVAT as a potential mediator for the inflammatory and vascular function component in obesity linked to CVD. However, knowledge of the relationship between aPVAT and arterial stiffness is limited. There are limited studies examining the association between aPVAT and arterial stiffness in population-based cohorts and the FHS consists of a predominantly white population and it is hard to generalize their findings to other races.

The objective of this paper is to investigate the association between aPVAT and arterial stiffness in a population-based biracial cohort of middle-aged men. We hypothesize that greater aPVAT volume will be associated with higher cfPWV, a well-established measure of arterial

stiffness, in middle-aged men. In two multi-racial studies, the African-American group had less ectopic cardiovascular fat and less PVAT compared to other race groups including whites (37,68). Thus, we hypothesize that the association between aPVAT and cfPWV will be stronger in white men compared to African-American men.

Table 1. Overview of Studies of aPVAT and CVD Risk Factors and Vascular Outcomes

Population & Author (Year)	Title	Primary Outcome	Results
Framingham Heart Study n=2735 Britton et al (2014)	Thoracic Periaortic and Visceral Adipose Tissue and Their Cross-sectional Association with Measures of Vascular Function	flow-mediated dilation (FMD), hyperemic mean flow velocity, peripheral arterial tone ratio, cfPWV, forward wave amplitude, and mean arterial pressure	TAT[1] and VAT[2] were associated with multiple vascular function measures after multivariable adjustment. After BMI adjustment, TAT[1] remained negatively associated with hyperemic mean flow velocity p=0.03, peripheral arterial tone p=0.02 and 1000/cfPWV p=0.0009. Associations of TAT[1] with vascular function were attenuated after VAT[2] adjustment p>0.06
Framingham Heart Study Offspring n=1067 Lehman et al. (2010)	Peri-aortic Fat, Cardiovascular Disease Risk Factors, and Aortic Calcification: The FHS	Thoracic abdominal aortic calcification and coronary artery calcification	Thoracic aortic fat was associated with abdominal aortic calcification p=0.005 and coronary artery calcification p=0.001 after adjusting for CVD[6] risk factors and VAT[2]
Framingham Heart Study n=3246 Britton et al. (2012)	Prevalence, Distribution, and Risk Factor Correlates of High Thoracic Periaortic Fat	Cardiometabolic risk factors: BMI, WC, glucose, HTN, LDL-C, smoking, and metabolic syndrome	Among individuals with normal VAT[2], high TAT[1] compared with normal TAT[1] was associated with a more adverse cardiometabolic profile. These individuals were older and had a higher prevalence of the majority of cardiometabolic risk factors, including a higher prevalence of metabolic syndrome. Similar findings when adjusted for BMI. The presence of high TAT[1] was associated with prevalent CVD[6] p=0.004

Table 1 Continued

Framingham Heart Study n=100 Schlett et al (2013)	Novel measurements of periaortic adipose tissue in comparison to anthropometric measures of obesity, and abdominal adipose tissue	SAT, VAT	Abdominal periaortic adipose tissue and TAT[1] highly correlated with VAT[2] and moderately with SAT[3], waist circumference, and BMI
PREDICT study n=385 Brinkley et al (2014)	Periaortic Fat and Cardiovascular Risk: A comparison of High-Risk Older Adults and Age-Matched Healthy Control	Cardiovascular Risk factors	Individuals with risk factors for a cardiovascular event had significantly greater periaortic fat volumes compared to those without risk factors, when matched one-on-one.
Framingham Offspring and Third Generation n=3001 Thanassoulis et al (2012)	Periaortic Adipose tissue and Aortic Dimensions in the FHS	Aortic dimensions	Thoracic periaortic fat was associated with higher aortic dimensions both the thorax and abdomen $p<0.001$ The association persisted after adjustment for age, sex, and cardiovascular risk factors including BMI and VAT[2]
Framingham Heart Study Offspring cohort n=1205 Fox et al (2010)	Peri-Aortic Fat Deposition is associated with Peripheral Arterial Disease	Peripheral Arterial Disease	Per 1 std increase in peri-aortic fat, the OR=1.52; the results strengthened with additional adjustment for BMI or visceral abdominal fat
Mevlana University hospital patients n=178 Akyurek et al (2014)	Thoracic periaortic adipose tissue in relation to cardiovascular risk in type 2 diabetes mellitus	cardiovascular risk in type 2 diabetes mellitus	Patients with type 2 diabetes mellitus, TAT[1] volume, fasting blood glucose, total cholesterol, triglyceride, and LDL cholesterol levels were significantly higher compared with the control (nondiabetic) group

Table 1 Continued

Pittsburgh Lupus Registry n=287 Shields et al (2017)	Perivascular adipose tissue of the descending thoracic aorta is associated with systemic lupus erythematosus and vascular calcification in women	Aortic calcification	Women with SLE[7] had greater median aPVAT[5] and greater median AC than health control women. Total aPVAT[5] remained significantly associated with SLE after adjusting for CVD[6] risk factors. SLE[7] aPVAT[5] associated with aortic calcification after adjusting for circulating inflammatory markers and coronary artery calcification
SWAN Heart n=524 El Khoudary et al (2018)	Cardiovascular Fat in Women at Midlife: effects of race, overall adiposity and central adiposity: The SWAN Cardiovascular Fat Study	Cardiovascular Fat: epicardial, paracardial, total heart, and perivascular fat	After adjusting for age, study site, menopausal status, hypertension, diabetes, alcohol consumption, and physical activity, black women had less PVAT[4] than white women. Significant remained same after adjusting for BMI and SAT[3].
Fleenor et al (2014)	Superoxide signaling in perivascular adipose tissue promotes age-related artery stiffness	Arterial stiffness (aPWV)	Compared with aortic segments from old mice with absence of PVAT[4] media, old mice with presence of PVAT[4] media had greater intrinsic stiffness ($p<0.05$)

[1] TAT= Thoracic periaortic adipose tissue
[2] VAT= Visceral adipose tissue
[3] SAT = Subcutaneous adipose tissue
[4] PVAT= Perivascular adipose tissue
[5] aPVAT= periaortic adipose tissue
[6] CVD = Cardiovascular disease
[7] SLE = Systemic lupus erythematosus

2.0 Methods

2.1 Study Population

The ERA-JUMP (Electron-Beam Tomography Risk Factor Assessment Among Japanese and U.S. Men in the Post-World War II Birth Cohort) is a population-based cohort study of men who were 40-49 years old and free from CVD, type-1-diabetes, or other severe diseases at the time of recruitment, which was between 2002-2006 as previously described (69). These participants were brought back after average of 5 years later for follow-up assessment. The study primarily collected participants' diet and adiposity measurements to observe differences between races. A total of 1,355 men were randomly selected at 4 centers: Allegheny County, Pennsylvania, US, Kusatsu, Shiga, Japan, Ansan, South Korea, and Honolulu, Hawaii, US. A total of 417 men participated in Allegheny County, Pennsylvania, US, and this group consisted of 107 of African-American and 310 White men. The current study only includes participants from Allegheny County, Pennsylvania, where aPVAT was assessed. Of the 417 participants, 41 did not have cfPWV measurement at baseline, 30 of the participants' records were missing, 8 were unreadable, 1 did not have aPVAT measurement, 1 was missing most of the covariates, 2 were missing VAT, 1 had BMI higher than 50, 3 had CRP higher than 40 or missing, and 2 had aorta length that was less than 0. In this study, valid cfPWV measures were defined as less than 2000 cm/s (70). Therefore, the current analyses are limited to 324 participants from Allegheny County site of ERA-JUMP. The study was approved by the institutional review boards of the University of Pittsburgh, Pittsburgh, PA. Written informed consent was received from all participants.

2.2 Adipose Tissue Related Measures

Participants' periaortic adipose tissue (aPVAT) was measured using images acquired from Electron Beam Computed Tomography (EBCT) scans using a c-150 Ultrafast CT scanner (GE Imatron, San Francisco, CA, USA). EBCT images of the descending aorta (6mm-thick transverse images) were acquired and used to quantify aPVAT using Slice-O-Matic 4.3 software (Tomovision Quebec, Canada) at the University of Pittsburgh Ultrasound Research Lab. The pulmonary bifurcation was used as a marker of the proximal boundary and first lumbar vertebrae was used as a marker of the distal boundary of the descending aorta. The vertebral foramen was used as a marker of the posterior border and the anterior borders included horizontal line through the left bronchus (67). The first proximal image includes the carina, also known as the pulmonary bifurcation, and each image is quantified until pedicles of L1. As the image progress distally from the carina, the anterior border eventually be the interior border of the crus of the diaphragm. Aorta length was estimated from table position number at first to last CT slice. The length of the descending aorta within the anatomic landmarks many vary (71). The adipose tissue area was calculated by the range of attenuation values for adipose tissue, within the -190 and -30 Hounsfield units. aPVAT volume (cm^3) was calculated by summing the areas and multiplying by the slice thickness, 6mm. Within reader Spearman correlation for PVAT were 0.99, indicating excellent reproducibility (67).

Subcutaneous abdominal adipose tissue (SAT) and visceral adipose tissue (VAT) area was measured using a 6-mm transverse CT image at the level between the fourth and fifth lumbar vertebrae obtained from a GE-Imatron C150 scanner (GE Medical Systems, South San Francisco, U.S.). Adipose tissue was measured using the image analysis software AccuImage (AccuImage Diagnostics, San Francisco, CA) and distinguished from other tissues in the CT image using a

range of -160 to 0 Hounsfield Units. A separation line was manually drawn using a cursor on abdominal wall musculature in continuity with fascia of the para-spinal muscles to determine respective areas of VAT and SAT (72). Area of SAT was calculated from total abdominal adipose tissue minus VAT. All CT images were analyzed at the Cardiovascular Institute in University of Pittsburgh by trained readers with inter-class correlation coefficient of 0.99 for SAT and 0.99 for VAT (72).

2.3 Pulse Wave Velocity

As previously described, trained vascular sonographers from the Ultrasound Research Laboratory in University of Pittsburgh performed a measurement of pulse wave velocity (PWV) (73). PWV was measured using a noninvasive, automated waveform analyzer (VP2000, Omron, Japan) at baseline and follow-up visits (74). After resting for 10 minutes in a supine position, a participant had occlusion and monitoring cuffs placed around both light-clothing or bare arms and ankles. ECG electrodes placed on both wrists and a phonocardiogram, a microphone for detecting heart beats, was placed on the left edge of sternum. The cuffs were connected to a plethysmographic sensor, which determined volume pulse form and an oscillometric pressure sensor that measured blood pressure (BP). Sonographers palpated the left femoral artery and the left carotid artery and placed the handheld tonometers over the two pulse areas to obtain femoral and carotid pulse waveforms simultaneously. A foot pedal was used to start the recording of data being collected two times for each participant, the values were average. PWV by time-phase analysis using volume waveforms of respective arteries was calculated as the path length between arterial sites divided by time delay between the foot of the respective waveform. For cfPWV, the

path length was measured in cm over the surface of the body with tape measure: from the suprasternal notch to the sampling site on the left common carotid artery (SN-CCA), from the suprasternal notch to the inferior edge of the umbilicus (SN-umbilicus), and from the interior edge of the umbilicus to the sampling site on the left common femoral artery (umbilicus-FA). The carotid to femoral length was calculated by subtracting the SN-CCA from the sum of SN-umbilicus and umbilicus-FA. Furthermore, the path length for the heart-femoral (hfPWV) and the brachial-ankle (baPWV) segments were calculated using the following height-based formulae: hfPWV: 0.5643xheight; baPWV: (0.8129xheight+12.328) - (0.2195xheight-2.0734). Data were collected two times for each participant and the values were averaged. The same protocol was utilized for PWV measurements in the follow-up visit. Intra-class correlation coefficient was 0.84 for cfPWV, 0.86 for hfPWV, and 0.97 for baPWV (75).

2.4 Covariates

All participants completed a lifestyle questionnaire, physical examination, and laboratory assessment. The questionnaire contained questions about smoking and drinking status, medication, and race (76). Smoking was assessed as current, former, or never and alcohol consumption was whether participant drank any alcoholic beverage (beer, wine, liquor etc) ≥ 2 times per week or < 2 per week. Uses of hypertensive, diabetic, and lipid-lowering medication were reported yes or no. Body weight and height were measured while the participant wore light clothing without shoes and calculated BMI as weight in kg / (height in meter)^2. Waist circumference (cm) was measured at the level of the umbilicus while participant was standing.

Blood sample was collected after a 12-hour fasting during the clinic visit. The samples were stored at -80℃ and sent to a lab to determine serum levels of lipids, fasting glucose, insulin, and hsCRP. The serum lipids were determined using standardized method by the Centers for Disease Control and Prevention, including total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides. Glucose was quantified using an enzymatic assay, insulin using a radio-immunoassay (Linco Research Inc., St. Charles, US), and hsCRP using a calorimetric-competitive-enzyme-linked-immuno-sorbent assay. HOMA-IR was calculated using following formula: (fasting insulin x fasting glucose)/22.5.

Blood pressure was measured in the right arm of the comfortably seated participants after emptying their bladder and seated quietly for 5 minutes, using an automated sphygmomanometer and appropriate-sized cuff (BP-8800m Colin Medical Technology, Komaki, Japan) (69). The average of two measurements was used in this study. Hypertension was defined as systolic BP ≥ 140 mmHg, diastolic BP ≥ 90mmHg or use of antihypertensive medications, which was reported as yes/no. Diabetes mellitus was defined by fasting serum glucose level ≥ 7mmol/L or use of antidiabetic medications, which reported as yes/no.

2.5 Statistical Analysis

Descriptive statistics in the overall group and by race were calculated for demographic, exposure and outcome variables, CVD risk factors, inflammatory marker, and other adipose tissue measures, such as VAT and SAT for baseline and follow-up visit. The primary “exposure” of interest was aPVAT. The primary outcome variable was cfPWV. Both variables were tested for normality. The white and African-American groups were compared among many covariates: age,

heart rate, waist circumference, BMI, smoking status, alcohol drinking status, exercise, hypertension, diabetes, medications, blood pressure, cholesterol, triglycerides, glucose, insulin, CRP, aorta length, VAT, SAT, and PWV. The t-test was used to determine difference between the two racial groups for continuous measures. The Chi-squares test was used for categorical measures, unless the expected count in one or more cells was fewer than 5, then the Fisher-exact test was used. Multiple linear regressions were used to test the significance of covariate-adjusted relations between aPVAT and cfPWV. Variables for building models were considered if they were known to be associated with exposure and the outcome. For these variables, those with Pearson correlations $p<0.1$ were included in the model. Adiposity measurements, such as BMI, VAT, and aPVAT, were tested for multicollinearity. If the variance inflation factor was larger than 10, the variable was considered highly correlated with other variables and dropped from the model.

First, aPVAT and cfPWV association was examined in an unadjusted model. The first multivariable model was adjusted for age, aorta length, and race, variables known to be associated with both the exposure and outcome. The second multivariable models were a continuous build-up from model 1 but it included traditional CVD risk factors such as, systolic BP, triglyceride, HOMA-IR, physical activity, and alcohol consumption variables. Lastly, the third multivariable models, building on the second multivariable model, explored different pathways. The models were categorized into 3 different paths. The first path adjusted for CT measured visceral adipose tissue. The second path adjusted for waist circumference, a surrogate clinical measurement for visceral adiposity. The last path adjusted for traditional clinical measurement of overall adiposity, BMI. For each of these pathways, a 4^{th} and 5^{th} model was run additionally adjusting for CRP and medications, respectively. The models were run again with aPVAT and race interaction term and

aPVAT and VAT tertile interaction term separately to determine if race or VAT tertiles moderates the association between aPVAT and cfPWV.

In secondary analysis, hfPWV and baPWV were considered as an outcome using a similar approach to model building as described for cfPWV. In exploratory analysis to examine if aPVAT predicts cfPWV in average of 5 years later, the association between baseline aPVAT and follow-up visit cfPWV was explored while adjusting for baseline cfPWV. Next, baseline visit cfPWV was subtracted from follow-up visit to see if the change of cfPWV was associated with aPVAT, adjusting for covariates. Then, two sensitivity analyses were run. In the first sensitivity analyses, observations with CRP higher than 10 were deleted to see if the result of association between cfPWV and aPVAT were affected by acute inflammation. In the second sensitivity analyses, we excluded observations greater than 3 standard deviations from the mean of cfPWV to see if the association is driven by observations with extreme high/low of cfPWV values. All analyses were performed with SAS version 9.3 (SAS Institute, Cary, NC) for Windows. P-values of <0.05 were considered statistically significant for the main analysis.

3.0 Results

3.1 Study Population Characteristics

Overall and race-specific characteristics of study participants, and mean values for outcomes and exposures are summarized (Table 2). Mean age of the study population was 44.9±2.8 and 77.5% were white men. African-American men were more likely to be current smokers ($p<0.0001$), hypertensive ($p=0.006$), and have higher CRP ($p=0.04$) compared with whites. Whites had significantly longer aorta length ($p=0.003$), more aPVAT ($p=0.011$), and VAT ($p<0.0001$) compared with African-American men. Although all PWV trended towards being higher in African-American men compared with white men and baPWV was significantly higher, the main outcome, cfPWV, did not differ by race (Figure 1).

Table 2. Participant Characteristics Overall and by Race

Clinical Characteristics*	**All (n=324)**	**Whites (n=251)**	**AA (n=73)**	**P-value**
Age (years)	44.9 (2.8)	44.9 (2.9)	45.0 (2.8)	0.94
Race, Black **N (%)**	73 (22.5)	-	-	
Smoking Status**,** **N (%)**				<0.0001
Never Smoked	227 (70.1)	189 (75.3)	38 (52.1)	
Past Smoker	59 (18.2)	47 (18.7)	12 (16.4)	
Current Smoker	38 (11.7)	15 (6.0)	23 (31.5)	
Alcohol Drinking Status, **N (%)** *≥ twice per week*	139 (42.9)	111 (44.2)	28 (38.4)	0.373
Exercise, **N (%)** *≥ one hour per week*	242 (74.7)	188 (74.9)	54 (74.0)	0.87
Hypertension, **N (%)**	58 (17.9)	37 (14.7)	21 (28.8)	0.006
Medication for Hypertension, **N (%)**	30 (9.3)	19 (7.6)	11 (15.1)	0.052
Medication for Lipid, **N (%)**	35 (10.8)	29 (11.6)	6 (8.2)	0.42
Diabetes, **N (%)**	11 (3.4)	6 (2.4)	5 (6.9)	0.08

Table 2 Continued

Medication for Diabetes, **N (%)**	1 (0.3)	0 (0.0)	1 (1.4)	0.23
Systolic Blood Pressure, (mmHg)	122.7 (12.4)	122.3 (11.6)	124.1 (14.8)	0.35
Diastolic Blood Pressure, (mmHg)	73.0 (9.4)	72.9 (8.8)	73.2 (11.3)	0.87
Heart Rate (beats/min)	64.5 (9.9)	63.9 (9.3)	66.5 (11.6)	0.08
Total Cholesterol (mg/dL)	211.8 (39.8)	211.8 (37.5)	211.7 (47.2)	0.98
HDL Cholesterol (mg/dL)	49.3 (14.1)	48.4 (12.9)	52.2 (17.4)	0.09
Triglycerides (mg/dL)	140.3 (72.5)	143.6 (73.7)	129.1 (67.5)	0.12
LDL Cholesterol, (mg/dL)	134.5 (35.1)	134.8 (33.1)	133.7 (41.3)	0.83
Glucose (mg/dL)	100.5 (11.4)	100.2 (9.7)	101.8 (15.9)	0.41
Insulin (μIU/mL)	14.4 (7.3)	14.7 (7.5)	13.5 (6.1)	0.18
HOMA-IR	63.3 (37.3)	66.3 (38.7)	62.1 (31.7)	0.35
C-reactive Protein (mg/L)	1.7 (2.6)	1.5 (2.4)	2.3 (3.1)	0.04
Adiposity Related Measures				
aPVAT (cm^3)	47.7 (19.4)	49.2 (19.5)	42.7 (18.2)	0.011
Aorta_length	180.8 (20.7)	183.1 (17.6)	172.9 (27.6)	0.003
Mean HU unit	-83.5 (4.3)	-83.8 (4.4)	-82.7 (3.8)	0.043
Waist Circumference (cm)	96.9 (10.8)	97.2 (10.3)	96.0 (12.6)	0.44
BMI (kg/m^2)	27.6 (4.0)	27.4 (3.7)	28.3 (5.0)	0.16
Visceral Adipose Tissue (cm^2)	155.9 (67.2)	165.8 (67.2)	121.9 (55.2)	<0.0001
Subcutaneous Adipose Tissue (cm^2)	242.5 (112.9)	240.5 (102.9)	249.2 (143.0)	0.63
Pulse Wave Velocity Measures				
Carotid Femoral PWV (cm/s)	864.3 (207.1)	861.6 (206.6)	873.8 (210.3)	0.66
Heart-Femoral PWV (cm/s)	896.5 (140.8)	889.3 (134.3)	921.1 (159.8)	0.09
Brachial-Ankle PWV (cm/s)	1329.6 (163.9)	1312.6 (152.1)	1388.4 (188.9)	0.002

*Continuous data presented as **mean (STD)** or **median (25^{th} percentile, 75^{th} percentile)**;
BMI, body mass index; aPVAT, periaortic adipose tissue; PWV, pulse wave velocity

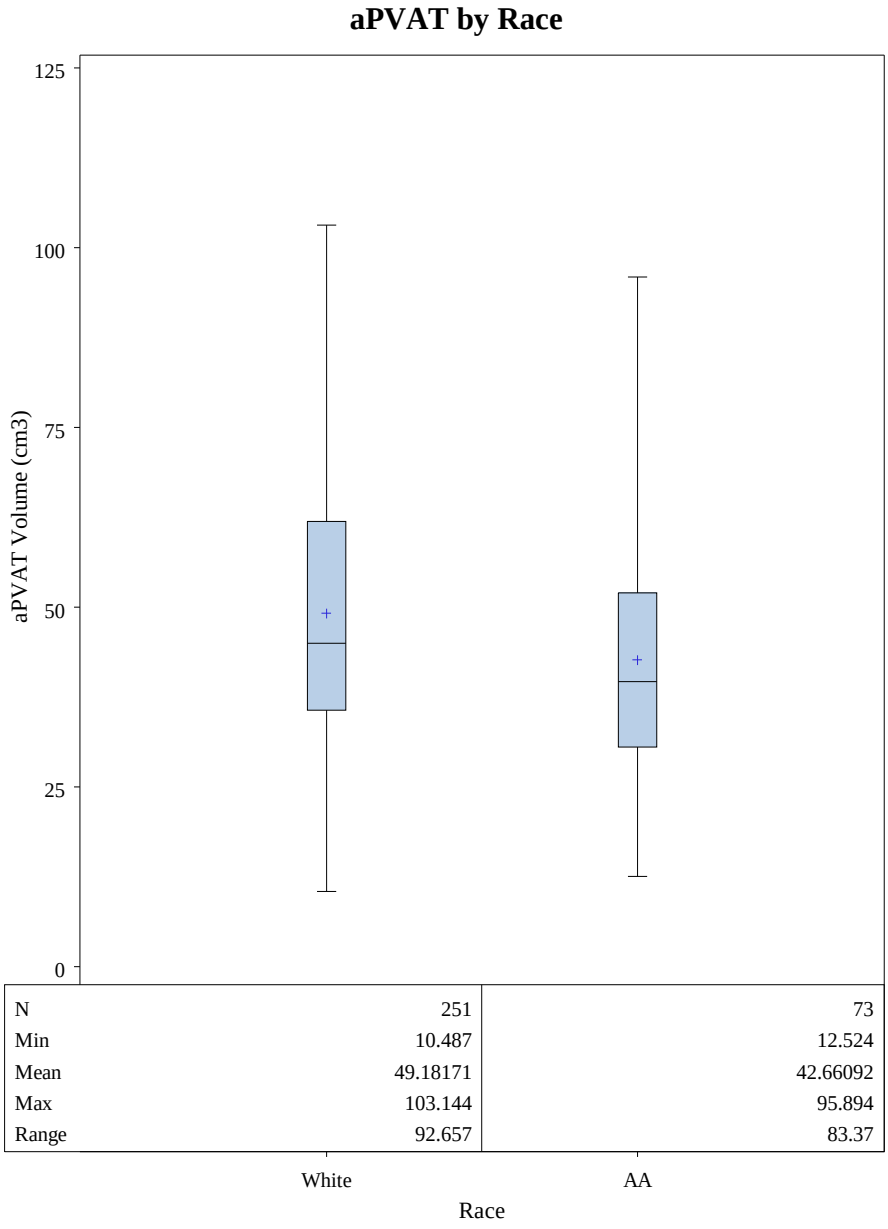

Figure 1. Distribution of aPVAT by Race

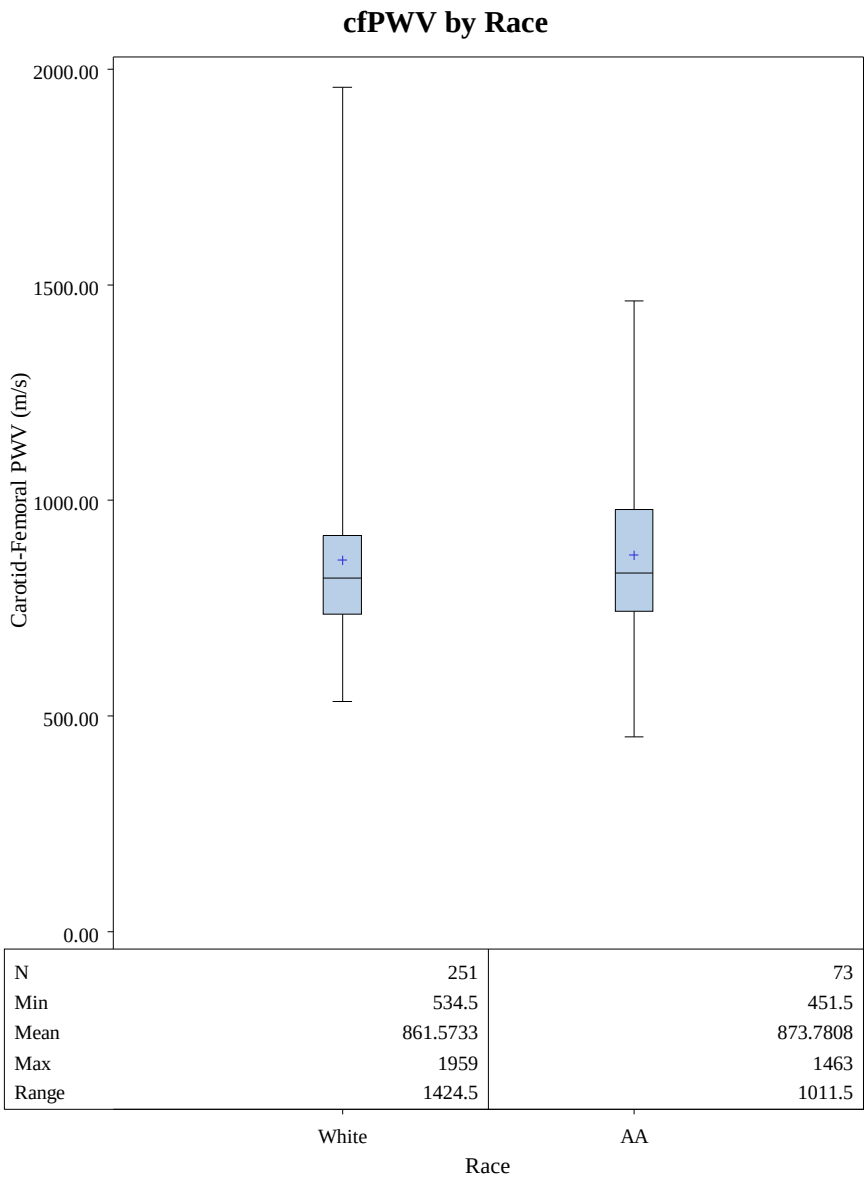

Figure 2. Distribution of cfPWV by Race

By the follow-up visit, only 16.3% of the population is African-American compared to 22.5% from the baseline (Table 3). Similar to baseline, African-American men were more likely to be current smokers (p=0.007), be hypertensive (p=0.026), and have more VAT compared to whites (p=0.004). White men had significantly higher insulin compared to African-American men

(p=0.009), which is different from baseline visit. PWV measures did not differ by race at follow-up. By the follow-up visit, more participants were taking medications for hypertension (19.1%), lipids (19.5%), and diabetes (2.3%). In addition, the follow-up visit participants had higher mean triglycerides (152.1 mg/dL), hfPWV (945.6 cm/s), and baPWV (1363.9 cm/s) compared to baseline participants. cfPWV was lower in the follow-up visit participants (860.1 cm/s).

Table 3. Participant Characteristics Overall and by Race in Follow-up Visit

Clinical Characteristics*	**All (n=215)**	**Whites (n=180)**	**AA (n=35)**	**P-value**
Age (years)	49.5 (2.9)	49.6 (2.9)	49.3 (3.0)	
Black Race, **N (%)**	35 (16.3)	-	-	
Smoking Status**,** **N (%)**				0.007
Never Smoked	158 (73.5)	189 (75.3)	38 (52.1)	
Past Smoker	37 (17.2)	47 (18.7)	12 (16.4)	
Current Smoker	20 (9.3)	15 (6.0)	23 (31.5)	
Alcohol Drinking Status, **N (%)** *≥ twice per week*	97 (45.1)	86 (47.8)	11 (31.4)	0.075
Exercise, **N (%)** *≥ one hour per week*	164 (76.3)	143 (79.4)	21 (60.0)	0.013
Hypertension, **N (%)**	59 (27.4)	44 (24.4)	15 (42.9)	0.026
Medication for Hypertension, **N (%)**	41 (19.1)	30 (16.7)	11 (31.4)	0.042
Medication for Lipid, **N (%)**	42 (19.5)	38 (21.1)	4 (11.4)	0.246
Diabetes, **N (%)**	18 (8.4)	15 (8.3)	3 (8.6)	0.256
Medication for Diabetes, **N (%)**	5 (2.3)	2 (1.1)	3 (8.6)	0.032
Systolic Blood Pressure, (mmHg)	125.6 (12.8)	125.6 (11.6)	125.7 (13.0)	0.953
Diastolic Blood Pressure, (mmHg)	76.6 (8.3)	76.4 (8.8)	77.3 (8.4)	0.592
Heart Rate (beats/min)	65.8 (10.0)	66.0 (9.9)	66.5 (11.6)	0.521
Total Cholesterol (mg/dL)	212.6 (39.1)	213.5 (37.5)	208.2 (46.8)	0.535
HDL Cholesterol (mg/dL)	50.1 (12.4)	50.0 (12.9)	50.5 (12.4)	0.811
Triglycerides (mg/dL)	152.1 (117.5)	154.1 (73.7)	141.5 (102.2)	0.519
LDL Cholesterol, (mg/dL)	132.5 (34.0)	133.3 (33.1)	128.4 (38.8)	0.488
Glucose (mg/dL)	108.1 (20.3)	107.6 (9.7)	110.8 (28.0)	0.514
Insulin (μIU/mL)	13.9 (7.0)	14.3 (7.5)	12.0 (3.8)	0.009
HOMA-IR	69.3 (48.4)	71.2 (51.5)	59.8 (25.9)	0.054
C-reactive Protein (mg/L)	1.7 (2.6)	1.7 (2.7)	1.8 (1.7)	0.748
Adiposity Related Measures				
Waist Circumference (cm)	99.4 (11.0)	99.5 (10.8)	99.2 (12.0)	0.895
BMI (kg/m^2)	28.2 (3.9)	28.0 (3.7)	29.4 (4.9)	0.112

Table 3 Continued

Visceral Adipose Tissue (cm^2)	151.9 (57.7)	156.1 (59.3)	130.4 (43.4)	0.004
Subcutaneous Adipose Tissue (cm^2)	232.7 (85.0)	230.3 (80.9)	245.0 (104.1)	0.435
Pulse Wave Velocity Measures				
Carotid Femoral PWV (cm/s)	860.1 (180.4)	859.0 (176.5)	865.8 (201.7)	0.853
Heart-Femoral PWV (cm/s)	945.6 (142.8)	945.2 (142.3)	947.2 (147.7)	0.941
Brachial-Ankle PWV (cm/s)	1363.9 (174.4)	1361.0 (169.1)	1378.7 (201.5)	0.629

*Continuous data presented as **mean (STD)** or **median (25^{th} percentile, 75^{th} percentile)**;
BMI, body mass index; aPVAT, periaortic adipose tissue; PWV, pulse wave velocity

3.2 Association Between aPVAT and cfPWV

In univariate linear regression, aPVAT was positively associated with cfPWV (2.28[1.13, 3.42], p=0.0001) and this association remained significant after adjusting for aorta length, age, and race (Table 4). The model was expanded to include CVD risk factors: SBP, triglyceride, HOMA-IR, exercise, and alcohol drinking status, in which aPVAT remained associated with cfPWV (1.74[0.53, 2.94], p=0.005). In the first path adjusting for VAT, the aPVAT association with cfPWV was attenuated (1.35[-0.02, 2.73], p=0.054) and became strongly associated when further adjusted for CRP (1.43 [0.05, 2.81], p=0.042) and medications (1.53[0.14, 2.91], p=0.031). In the second path, aPVAT was significantly associated with cfPWV adjusting for WC (1.41[0.08, 2.75], p=0.038). When the model was expanded to adjust for CRP and medication, aPVAT effect became stronger (1.71[0.35, 3.07], p=0.014). When adjusted for BMI, the aPVAT association was attenuated (1.26[-0.01, 2.53], p=0.052) but strengthened when the model further adjusted for both CRP and medications (1.59[0.27, 2.90], p=0.018). As for covariates, in the final model (model 5) in addition to aPVAT, aorta length, SBP, and alcohol were significantly associated with cfPWV (Appendix Table 1). Within the VAT tertiles, the association between aPVAT and cfPWV does

become stronger the higher the tertiles (Figure 4) but the interaction term of aPVAT and VAT tertiles is not associated with cfPWV (data not shown).

Table 4. Multiple Linear Regression Models of the Association Between aPVAT and cfPWV

Models	**aPVAT**	
	β (95% C.I.)	***P-value***
Unadjusted	2.28 (1.13, 3.42)	0.0001
Model 1	2.59 (1.41, 3.77)	<0.0001
Model 2	1.74 (0.53, 2.94)	0.005
Models adjusting for VAT		
Model 3a	1.35 (-0.02, 2.73)	0.054
Model 4a	1.43 (0.05, 2.81)	0.042
Model 5a	1.53 (0.14, 2.91)	0.031
Models adjusting for WC		
Model 3b	1.41 (0.08, 2.75)	0.038
Model 4b	1.54 (0.20, 2.88)	0.025
Model 5b	1.71(0.35, 3.07)	0.014
Models adjusting for BMI		
Model 3c	1.26 (-0.01, 2.53)	0.052
Model 4c	1.42 (0.13, 2.70)	0.031
Model 5c	1.59 (0.27, 2.90)	0.018

Model 1: Aorta length + age + race
Model 2: Model 1 + SBP + triglyceride + HOMA-IR + physical activity + alcohol consumption
Model 3: Model 2 + adiposity measure
Model 4: Model 3 + CRP
Model 5: Model 4 + Hypertensive Medications + Lipid Medications + Diabetes Medications

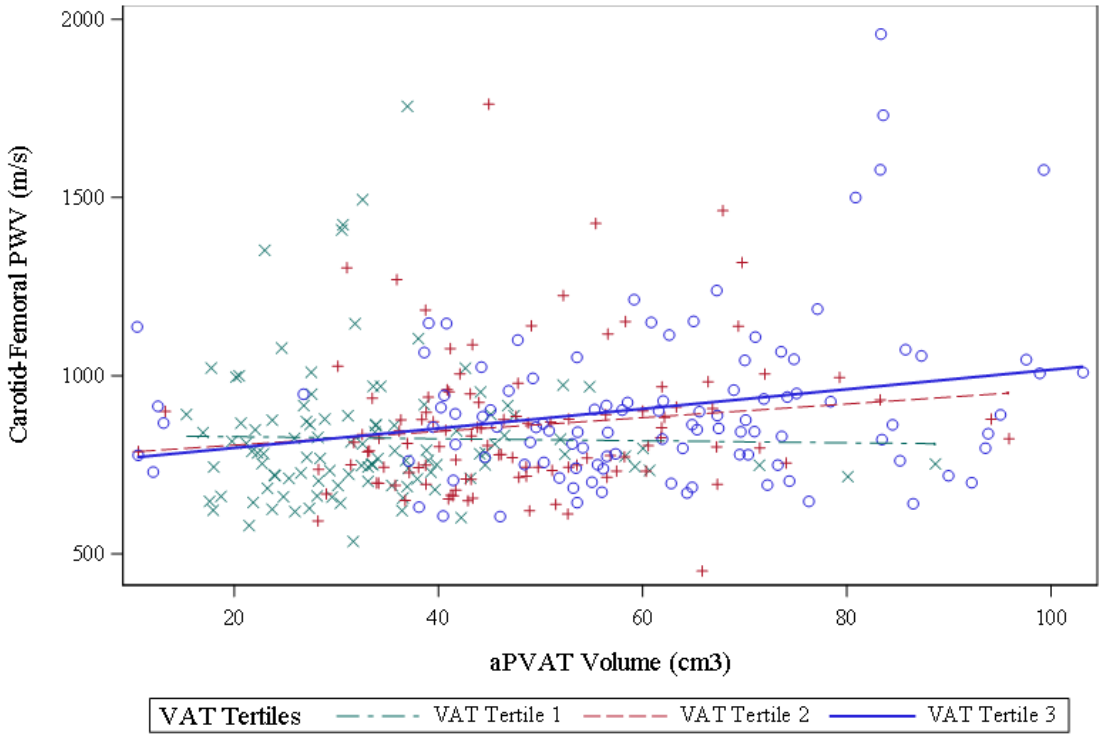

Figure 3. Linear Regression Between aPVAT and cfPWV by VAT Tertiles

3.3 Race Interaction

The regression line suggested that race did not moderate the association between aPVAT and cfPWV (Figure 3). This is confirmed in the multivariable linear regression. aPVAT and race interaction term was not significant in all the models with cfPWV as an outcome (Table 5). Yet, aPVAT alone was associated with cfPWV in unadjusted (3.79[0.13, 7.44], p=0.042) and adjusted for aorta length, age, race, and aPVAT and race interaction term (3.72[0.05, 7.37], p=0.046). Similar to cfPWV, aPVAT and race interaction term was not associated with all the models with

hfPWV as an outcome (data not shown). As for baPWV as an outcome, neither aPVAT nor the race interaction term was associated (data not shown).

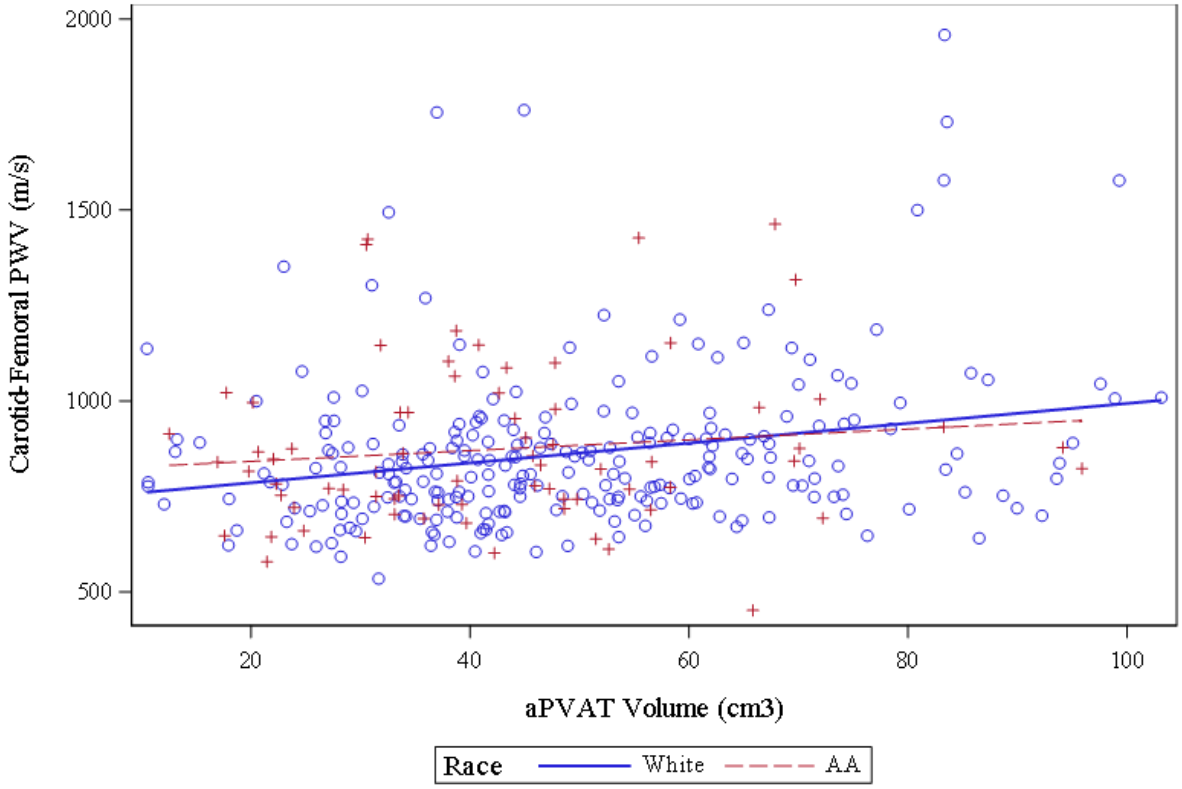

Figure 4 Linear Regression Between aPVAT and CfPW by Race

Table 5 Multiple Linear Regression Models of the Association Between aPVAT and cfPWV Including aPVATxRace Interaction Term

Models	**aPVAT**		**Race * aPVAT**	
	β (95% C.I.)	***P-value***	***β (95% C.I.)***	***P-value***
Unadjusted	3.79 (0.13, 7.44)	0.042	-1.19 (-4.08, 1.70)	0.418
Model 1	3.72 (0.06, 7.37)	0.046	-0.94 (-3.84, 1.95)	0.522
Model 2	2.69 (-0.80, 6.17)	0.130	-0.74 (-3.48, 2.00)	0.597
Models adjusting for VAT				
Model 3a	2.23 (-1.34, 5.80)	0.221	-0.68 (-3.42, 2.07)	0.628
Model 4a	2.25 (-1.31, 5.82)	0.214	-0.64 (-3.37, 2.10)	0.647
Model 5a	2.55 (-1.01, 6.12)	0.160	-0.80 (-3.53, 1.94)	0.566
Models adjusting for WC				
Model 3b	2.59 (-0.90, 6.07)	0.145	-0.95 (-3.71, 1.81)	0.499
Model 4b	2.73 (-0.75, 6.22)	0.124	-0.97 (-3.73, 1.79)	0.490
Model 5b	3.07 (-0.42, 6.57)	0.085	-1.11 (-3.86, 1.65)	0.431
Models adjusting for BMI				
Model 3c	2.32 (-1.16, 5.80)	0.190	-0.83 (-3.56, 1.90)	0.551
Model 4c	2.46 (-1.02, 5.94)	0.165	-0.82 (3.54, 1.90)	0.554
Model 5c	2.79 (-0.71, 6.29)	0.117	-0.95 (3.67, 1.78)	0.495

Model 1: Aorta length + age + race
Model 2: Model 1 + SBP + triglyceride + HOMA-IR + physical activity + alcohol consumption
Model 3: Model 2 + adiposity measure
Model 4: Model 3 + CRP
Model 5: Model 4 + Hypertensive Medications + Lipid Medications + Diabetes Medications

3.4 Secondary Analysis: Association Between aPVAT and hfPWV and baPWV

In univariate linear regression, aPVAT was positively associated with hfPWV (1.30[0.52, 2.09], p=0.001) and this association remained significant after adjusting for aorta length, age, and race (1.50[0.70, 2.30], p=0.0003) (Table 6). The model was expanded to include CVD risk factors: SBP, triglyceride, HOMA-IR, exercise, and alcohol drinking status, in which aPVAT remained marginally associated with hfPWV (0.72[-0.05, 1.49], p=0.066). aPVAT association with hfPWV was attenuated after adjusting for adiposity measurements and this effect became weaker when further adjusted for CRP and medications across all paths.

In univariate linear regression, aPVAT was marginally associated with baPWV (0.90[-0.03, 1.82], p=0.057) and this association became stronger after adjusting for aorta length, age, and race (0.90[0.10, 1.96], p=0.031) (Table 6). In the model including CVD risk factors: SBP, triglyceride, HOMA-IR, exercise, and alcohol, the aPVAT was no longer associated with baPWV (-0.32[-1.16, 0.53], p=0.462), even after further adjusting for adiposity measures.

Table 6. Multiple Linear Regression Models of the Association Between aPVAT and hfPWV and baPWV *

Models	**hfPWV as an outcome**		**baPWV as an outcome**	
	β (95% C.I.)	***P-value***	***β (95% C.I.)***	***P-value***
Unadjusted	1.30 (0.52, 2.09)	0.001	0.90 (-0.03, 1.82)	0.057
Model 1	1.50 (0.70, 2.30)	<0.001	1.03 (0.10, 1.96)	0.031
Model 2	0.72 (-0.05, 1.49)	0.066	-0.32 (-1.16, 0.53)	0.462
Models adjusting for VAT				
Model 3a	0.56 (-0.32, 1.44)	0.210	-0.85 (-1.81, 0.11)	0.082
Model 4a	0.60 (-0.28, 1.48)	0.182	-0.83 (-1.79, 0.14)	0.093
Model 5a	0.65 (-0.23, 1.54)	0.147	-0.82 (-1.78, 0.14)	0.094
Models adjusting for WC				
Model 3b	0.52 (-0.32, 1.37)	0.224	-0.40 (-1.34, 0.53)	0.398
Model 4b	0.59 (-0.26, 1.44)	0.176	-0.38 (1.33, 0.56)	0.425
Model 5b	0.69 (-0.18, 1.56)	0.118	-0.30 (-1.24, 0.65)	0.539
Models adjusting for BMI				
Model 3c	0.60 (-0.21, 1.41)	0.150	-0.31 (-1.22, 0.59)	0.493
Model 4c	0.67 (-0.15, 1.49)	0.111	-0.30 (-1.21, 0.62)	0.523
Model 5c	0.78 (-0.06, 1.62)	0.070	-0.20 (-1.12, 0.72)	0.671

* β, 95% Confidence Interval, and p-value of aPVAT are reported
Model 1: Aorta length + age + race
Model 2: Model 1 + SBP + triglyceride + HOMA-IR + physical activity + alcohol consumption
Model 3: Model 2 + adiposity measure
Model 4: Model 3 + CRP
Model 5: Model 4 + Hypertensive Medications + Lipid Medications + Diabetes Medications

3.5 Secondary Analysis: aPVAT Predicting cfPWV in Follow-up visit

In multiple linear regression, aPVAT predicted follow-up visit cfPWV adjusting for baseline cfPWV (p=0.001). aPVAT remained significant after adjusting for aorta length, age, race, and traditional CVD risk factors (1.42[0.20, 2.64], p=0.028) (Table 7). Although, this association was attenuated after adjusting for follow-up visit covariates (1.18[-0.08, 2.44], p=0.067). In the models with baseline covariates, aPVAT remained marginally significant when adjusting for VAT, CRP, and medications (1.32[-0.04, 2.69], p=0.058). This association was attenuated when adjusting for follow-up visit covariates (1.11[-0.26, 2.47], p=0.112). In linear regression with change of cfPWV between two visits as an outcome, aPVAT was not significant in any of the models (data not shown).

Table 7. Multiple Linear Regression Models of the Association Between Baseline aPVAT and Follow-up Visit cfPWV*

Models	**Adjusting for Baseline Covariates** (n=215)		**Adjusting for Follow-up Covariates** (n=215)	
	β (95% C.I.)	***P-value***	***β (95% C.I.)***	***P-value***
Unadjusted	2.13 (0.92, 3.34)	0.001	2.19 (0.97, 3.41)	0.001
Model 1	1.99 (0.73, 3.25)	0.002	2.02 (0.76, 3.29)	0.002
Model 2	1.42 (0.20, 2.64)	0.028	1.18 (-0.08, 2.44)	0.067
Models adjusting for VAT				
Model 3a	1.35 (-0.007, 2.67)	0.051	0.99 (-0.38, 2.36)	0.156
Model 4a	1.34 (-0.002, 2.68)	0.050	1.08 (-0.28, 2.44)	0.118
Model 5a	1.32 (-0.04, 2.69)	0.058	1.11 (-0.26, 2.47)	0.112
Models adjusting for WC				
Model 3b	1.10 (-0.20, 2.40)	0.097	1.01 (-0.30, 2.32)	0.131
Model 4b	1.12 (-0.18, 2.43)	0.091	1.14 (-0.16, 2.44)	0.086
Model 5b	1.10 (-0.24, 2.44)	0.108	1.15 (-0.17, 2.46)	0.087
Models adjusting for BMI				
Model 3c	1.13 (-0.12, 2.38)	0.072	0.88 (-0.40, 2.17)	0.177
Model 4c	1.16 (-0.10, 2.42)	0.071	1.05 (-0.23, 2.32)	0.107
Model 5c	1.11 (-0.19, 2.42)	0.094	1.06 (-0.22, 2.35)	0.105

* β, 95% Confidence Interval, and p-value of aPVAT are reported
Model 1: Aorta length + age + race

Model 2: Model 1 + SBP + triglyceride + HOMA-IR + physical activity + alcohol consumption
Model 3: Model 2 + adiposity measure
Model 4: Model 3 + CRP
Model 5: Model 4 + Hypertensive Medications + Lipid Medications + Diabetes Medications

3.6 Sensitivity Analysis

After excluding participants with CRP values higher than 10, there was a total of 320 individuals remaining in the analyses (Table 8). The results were similar to the primary outcome analysis with slight increase in effect sizes and confidence intervals away from zero adjusting for adiposity variable models. In another sensitivity analysis, observations outside ± 3 standard deviations of cfPWV were deleted (Table 8) to examine whether extreme low and high values of cfPWV may be driving the association with aPVAT (n=316). Only in the unadjusted model (1.24[0.30, 2.19], p=0.01) and expanded model adjusting for aorta length, age, and race was aPVAT statistically significantly associated with cfPWV (1.64[0.68, 2.59], p=0.001).

Table 8. Sensitivity Analysis linear Regression Models *

Models	**cfPWV (CRP>10) (n=320)****		**cfPWV (± 3 stddev) (n=316)*****	
	β (95% C.I.)	***P-value***	***β (95% C.I.)***	***P-value***
Unadjusted	2.27 (1.11, 3.43)	0.0001	1.24 (0.30, 2.19)	0.010
Model 1	2.61 (1.41, 3.81)	<0.0001	1.64 (0.68, 2.59)	0.001
Model 2	1.86 (0.63, 3.09)	0.003	0.87 (-0.09, 1.83)	0.074
Models adjusting for VAT				
Model 3a	1.41 (0.02, 2.81)	0.047	0.40 (-0.69, 1.48)	0.471
Model 4a	1.46 (0.07, 2.85)	0.040	0.48 (-0.60, 1.56)	0.383
Model 5a	1.56 (0.16, 2.96)	0.030	0.50 (-0.58, 1.59)	0.361
Models adjusting for WC				
Model 3b	1.51 (0.16, 2.87)	0.028	0.49 (-0.57, 1.54)	0.363
Model 4b	1.59 (0.23, 2.95)	0.022	0.61 (-0.44, 1.67)	0.255
Model 5b	1.76 (0.38, 3.14)	0.012	0.71 (-0.36, 1.78)	0.192
Models adjusting for BMI				
Model 3c	1.34 (0.04, 2.63)	0.043	0.55 (-0.46, 1.56)	0.288

Table 8 Continued

Model 4c	1.44 (0.14, 2.74)	0.030	0.69 (-0.33, 1.71)	0.183
Model 5c	1.62 (0.28, 2.95)	0.018	0.78 (-0.26, 1.82)	0.139

* β, 95% Confidence Interval, and p-value of aPVAT are reported
** Excluding participants with CRP higher than 10
*** Excluding participants with ± 3 standard deviation from mean of cfPWV
Model 1: Aorta length + age + race
Model 2: Model 1 + SBP + triglyceride + HOMA-IR + physical activity + alcohol consumption
Model 3: Model 2 + adiposity measure
Model 4: Model 3 + CRP
Model 5: Model 4 + Hypertensive Medications + Lipid Medications + Diabetes Medications

4.0 Discussion

To our knowledge, this is one of the first studies to examine the association between thoracic periaortic adipose tissue (aPVAT) and carotid-femoral pulse wave velocity (cfPWV), a gold standard measurement of arterial stiffness, within a biracial cohort of U.S. men. As hypothesized, aPVAT volume was associated with cfPWV in middle-aged men independent of traditional CVD risk factors. After further adjusting for different adiposity measurements (VAT, WC or BMI), similar associations between aPVAT and cfPWV persisted. In contrast to our hypothesis, race did not appear to moderate the relationship between aPVAT and cfPWV. This study provides a better understanding of the potential effect of local adipose tissue on arterial stiffness and how race plays a role in the relationship between adipose tissue with stiffening of arteries.

These findings indicate that aPVAT and cfPWV association exists beyond CVD risk factors and other adiposity measurements. The Framingham Heart Study (FHS), a community-based study, showed similar results that aPVAT was associated with cfPWV after adjusting for traditional CVD risk factors (63). In our study, adjusting for waist circumference resulted in a larger effect size compared to adjusting for BMI. We have found that the effect estimate for aPVAT was the lowest when controlling for VAT. In addition, VAT did not moderate the relationship between aPVAT and cfPWV, which is consistent with FHS results (63). Collectively, these finding suggests that VAT does not fully account for the relationship between aPVAT and cfPWV, which means VAT may not a total surrogate marker for aPVAT. One alternative explanation for VAT not accounting for the relationship between aPVAT and cfPWV is that the measurement of aPVAT in the current study does not include abdominal aortic fat. A previous

study presented high correlation between abdominal aortic fat and VAT, which makes abdominal aortic fat as an important factor in VAT (64). Therefore, lack of inclusion of adipose tissue located on abdominal aorta may have precluded identification of an association between aPVAT and cfPWV beyond VAT. CRP was added into the model in an attempt to examine the role of inflammation in the relationship between aPVAT and cfPWV and CVD risk factor medications were added to control for other diseases that could affect the outcome. It is important to note that CRP is not a specific marker of inflammation, rather systemic. Controlling for CRP, considers for any other underlying disease participants may have at the time of study. Further adjusting for a marker of inflammation and CVD related medications, did not eliminate this association

In this cohort, African-American group had less aPVAT volume, which is consistent with a previous study in women (68). The Study of Women's Health Across the Nation (SWAN), a community-based-multisite study showed African-American women had less aPVAT compared to white women (68). The main outcome, cfPWV did not differ between the two races statistically but African-American group presented higher cfPWV compared to white group, which is consistent with the ARIC study, as well as other population-based studies (77). Despite the differences in aPVAT volume and cfPWV between race, the relationship between aPVAT and cfPWV was not moderated by race. One of the potential issues with race not moderating the relationship between aPVAT and cfPWV could partly be due to a small sample size, especially in the African-American men or perhaps the association is similar in both groups.

Previously, pulse wave velocity measured in other arterial sites have been used in population studies (75). These include heart-femoral pulse wave velocity (hfPWV), which is direct measurement of central aortic stiffness, and brachial-ankle pulse wave velocity (baPWV), a mixed measurement of central artery and peripheral artery which are known for its association with

cardiovascular risk factors (75). Both hfPWV and baPWV were higher in the African-American men compared to white men. aPVAT was associated with both hfPWV and baPWV but was stronger with hfPWV, which is expected because both aPVAT and hfPWV include the central aorta. Also, hfPWV and baPWV individually association with aPVAT were attenuated when adjusted for traditional CVD risk factors, which means that the relationship between aPVAT and hfPWV/baPWV may not exist beyond traditional cardiovascular risk factors and other adiposity measurements. As for baPWV findings, this potentially could mean that aPVAT has more local effect than systemic. Moreover, another study with same population indicated baPWV was significantly associated with cardiovascular risk factors compared to cfPWV, which could explain attenuation after adjusting for traditional risk factors (75).

In the exploratory analysis to examine if aPVAT predicted follow-up cfPWV, we found that the aPVAT modestly associated with follow-up visit of cfPWV when adjusting for traditional cardiovascular risk factors and baseline cfPWV but aPVAT no longer predicted follow-up visit cfPWV when adjusted for both baseline and follow-up adiposity measurements. aPVAT had larger effect size when adjusting for baseline adiposity measurements. This suggests that baseline adiposity is more important than follow-up for current cfPWV. However, there is a reason to believe that aPVAT predicting cfPWV in the follow-up visit is compromised given the 34% decrease in sample size at the follow-up visit. This large percentage of participants drop-out could create a bias, meaning only those who stayed healthy were able to follow-up in the study rather than having more generalized study population. The result of aPVAT predicting cfPWV might be different if more participants were followed-up because, with current sample size, the variability left in follow-up cfPWV may be too small to detect after adjusting for baseline cfPWV.

The study has several strengths including that the community study is based on biracial cohort. This is the first study to the best of our knowledge that looked at racial effect in association between aPVAT and cfPWV as well as evaluating if aPVAT predicts cfPWV in the follow-up visit. Additional strengths include the use of widely known adipose tissue measurements, and gold standard arterial stiffness measurement.

Several limitations within this study are worth noting. First given that the main analyses are based on a cross-sectional study design, this study cannot show temporal relationship between aPVAT and cfPWV, and thus we cannot infer cause and effect. No follow-up visit of aPVAT have been collected at this time. Therefore, the study cannot examine if the relationship persists in the follow-up visit. Lack of power due to a small sample size might precluded association of aPVAT and cfPWV as demonstrated in the sensitivity analysis excluding those with 3 standard deviation of cfPWV (n=316), where the association was attenuated when adjusting for adiposity measurements. The participants with 3 standard deviation were those with high cfPWV. Thus, the findings suggest that the association between aPVAT and cfPWV may be driven by high values of cfPWV or there is potential threshold effect of cfPWV. The threshold effect means that the relationship between aPVAT and cfPWV could only persists within a range of cfPWV instead of all cfPWV values. Although we did not attempt to account for the potential effects that inflammation may have in the aPVAT and cfPWV relationship, CRP adjustment does not exclude inflammation as a potential mediator because CRP, as a non-specific marker of systemic inflammation, does not particularly reflect local inflammation (14,15). A majority of the population was white (77.5%) and any extrapolation of the findings to other races must be made with caution. Also, study population with narrow age range and of men affects generalizability of the findings suggesting analysis needs to be done in women and or other race populations.

Obesity is a leading predisposing risk factor in CVD, which continues to be the leading cause of death in United States (1,4). Arterial stiffening is a one way to predict future adverse cardiovascular event, such as a stroke or heart failure. Generalized adiposity alone does not account for location of adipose tissue. More animal and populational studies are pointing towards location of adipose tissue, such as aPVAT, as an important factor contributing to adverse changes in vascular function and structure (56-61, 63). Therefore, it is crucial to better understand the mechanistic relationship between ectopic fat and arterial stiffness on the population level in different races and sexes. Next step is to extend these analyses to women and other races to see if the results are similar. Extensive epidemiological studies with larger cohorts are needed to further investigate the aPVAT contribution to vascular risk. Furthermore, this study only includes thoracic aortic fat so, next step would be to evaluate the relationship between aPVAT and cfPWV either by combining both abdominal and thoracic as total aPVAT or look at them separately to see if the relationship with cfPWV differs between two anatomically separated aortic fat.

In conclusion, middle-aged men with high thoracic periaortic fat volume are more likely to have stiffer arteries. The association between periaortic fat and arterial stiffness believed to reflect biologic pathways that could potentially predict altercation of future cardiovascular events. To our best of knowledge, this is the first paper to examine racial differences in aPVAT and cfPWV in men. Although middle-aged white men had more aPVAT volume and African-American men had higher cfPWV, there is not enough evidence to conclude that race moderates the relationship between aPVAT and cfPWV. Therefore, this study contributes to the existing literature on the association between localized effect of adipose tissue on arterial stiffening and includes novel findings in a biracial cohort of middle-aged men.

Appendix Supplemental Table

Appendix Table 1. Multiple Linear Regression Final Model of cfPWV Covariates*

Variables	VAT Model		WC Model		BMI Model	
	β (95% C.I.)	*P-value*	*β (95% C.I.)*	*P-value*	*β (95% C.I.)*	*P-value*
aPVAT	1.53 (0.14, 2.91)	0.031	1.71 (0.35, 3.07)	0.014	1.59 (0.27, 2.90)	0.018
Aorta Length	-1.48 (-2.56, -0.40)	0.007	-1.54 (-2.63, -0.46)	0.006	-1.48 (-2.56, -0.40)	0.007
Age	0.02 (-7.66, 7.69)	0.996	0.74 (-6.86, 8.34)	0.848	0.52 (-7.08, 8.12)	0.893
Race	23.81 (-32.21, 79.83)	0.404	8.73 (-44.61, 62.07)	0.748	-4.94 (-59.04, 49.16)	0.858
Systolic Blood Pressure	4.68 (2.84, 6.52)	<0.001	4.69 (2.84, 6.54)	<0.001	4.53 (2.66, 6.39)	<0.001
Trigly-ceride	0.07 (-0.26, 0.40)	0.682	0.11 (-0.21, 0.43)	0.509	0.07 (-0.26, 0.39)	0.647
HOMA-IR	-0.14 (-0.79, 0.52)	0.678	-0.16 (-0.83, 0.52)	0.646	-0.27 (-0.94, 0.41)	0.451
Exercise	-11.64 (-61.16, 37.88)	0.644	-15.25 (-64.65, 34.15)	0.544	-22.48 (-72.16, 27.05)	0.373
Alcohol	-53.77 (-97.86, -9.67)	0.017	-57.44 (-101.2, -13.7)	0.010	-56.49 (-100.0, -12.9)	0.011
Adiposity Measure	0.39 (-0.07, 0.85)	0.096	1.62 (-1.01, 4.26)	0.226	7.28 (0.30, 14.27)	0.041
Log C-reactive Protein	-20.25 (-43.56, 3.07)	0.086	-17.60 (-40.55, 5.34)	0.132	-18.51 (-41.30, 4.27)	0.106
Hyper-tensive Medica-tion	-43.01 (-119.9, 33.83)	0.272	-38.51 (-115.2, 38.16)	0.324	-32.74 (-109.07, 43.6)	0.399
Lipid Medica-tion	-11.39 (-81.20, 58.42)	0.748	-11.94 (-81.90, 58.02)	0.737	-9.52 (-79.20, 60.15)	0.788
Diabetes Medica-tion	337.68 (-49.33, 724.7)	0.078	301.90 (-87.8, 691.62)	0.128	269.73 (-120.1, 659.5)	0.174

* β, 95% Confidence Interval, and p-value of covariates for models 5a, 5b, and 5c are reported
Model 1: Aorta length + age + race
Model 2: Model 1 + SBP + triglyceride + HOMA-IR + physical activity + alcohol consumption
Model 3: Model 2 + adiposity measure
Model 4: Model 3 + CRP
Model 5: Model 4 + Hypertensive Medications + Lipid Medications + Diabetes Medications

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