

THE ASSOCIATION OF VISCERAL ADIPOSE TISSUE WITH PULSE PRESSURE
IN ADULTS WITHOUT CLINICAL CARDIOVASCULAR DISEASE

BY

MANISH JAIN

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Approved by:

David M. Herrington, M.D., M.H.S., Advisor

Examining Committee:

Barbara Nicklas, Ph.D., Committee Chair

Alain Bertoni, M.D.

Jeff Carr, M.D.

Jasmin Drivers, Ph.D.

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LIST OF ABBREVIATIONS

AFA= African- American

BP= Blood Pressure

CAU= Caucasians

CVD= Cardiovascular Disease

CHN= Chinese

CT= Computed Tomography

DEXA= Dual Energy X-ray Absorptiometry

ECG= Electrocardiogram

FFA= Free Fatty Acid

HDL= High density lipoprotein

HIS= Hispanics

MESA= Multi-Ethnic Study of Atherosclerosis

MRI= Magnetic Resonance Imaging

PP= Pulse Pressure

SD= Standard Deviation

TG= Triglycerides

US= Ultrasound

VLDL= Very low density lipoprotein

VF= Ventricular fibrillation

VT= Ventricular tachycardia

VAT= Visceral Adipose Tissue

VOI= Volume of Interest

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ABSTRACT

Manish Jain

THE ASSOCIATION OF VISCERAL ADIPOSE TISSUE (VAT) WITH PULSE PRESSURE IN THE MULTI ETHNIC STUDY OF ATHEROSCLEROSIS (MESA)

Thesis under the direction of David M. Herrington, M.D., M.H.S., Professor of Medicine

Background: Obesity and aortic stiffness (AS) contribute to cardiovascular disease (CVD). Central obesity as measured by VAT is thought to be a more specific measure of deleterious adiposity independent of body mass index (BMI). However, the nature of the relationship between VAT and AS in different sex and ethnic-specific subgroups is not well established.

Methods: The association between VAT and PP was assessed in a subset of MESA - a cohort of subjects aged 45-84 yrs without clinical CVD. VAT (mm^3) was estimated using 5, 3mm abdominal computed tomography slices centered at the L4–L5 level. PP (systolic– diastolic BP) - a surrogate for AS, and VAT variable were log transformed to reduce skewness. Multivariable linear regression models were used to describe the relationship between PP and VAT with and without adjustment for age, gender, heart rate, race, diabetes, and use of hypertensive medication.

Results: VAT and PP data were available for 398 subjects (mean age 65 yrs, 53% female, 57% Whites, 43% African-American (AA), mean Log VAT 2.23 ± 0.20 , mean Log PP 1.74 ± 0.13 mmHg). In univariate analysis, VAT was significantly associated with PP ($p = 0.0038$). Exploratory analysis revealed a significant interaction with gender ($p < 0.001$), with stronger associations in women than in men. In multivariate adjusted analyses stratified by gender there was a significant relationship between VAT and PP in women ($p = 0.0004$) but not in men ($p = 0.24$). This gender-specific relationship was stronger in African American women ($p = 0.0003$), than white women ($p = 0.02$).

Conclusions: Overall, VAT was associated with PP in this small sample of the MESA cohort. However, subgroup analysis revealed that the relationship between VAT and PP is modified by gender. This association was particularly strong in AA women. These data suggest that in AA women, VAT may have higher significance despite observations that AA women have lower VAT than other ethnicities. These data illustrate the need for more extensive and ethnic- and gender-specific research on the potential significance of VAT and its role in the pathophysiology of CVD.

CHAPTER 1

INTRODUCTION

I. The epidemic of obesity

Obesity has emerged as a global public health challenge. About one billion people in the world are overweight or obese. Recent data from CDC indicate that 40% of Americans over the age of 40 are obese as defined by BMI $>30 \text{ kg/m}^2$. Additionally, 66% of the US population is considered overweight (BMI >25). With the increase in childhood obesity, the global life expectancy in the United States is expected to fall for the first time in recent history (1). Among ethnic groups, African American and Hispanic women have the highest prevalences of obesity, at 50% and 40%, respectively (2) and unfortunately, these ethnic minorities are considered to be at higher risk for both cardiovascular disease and mortality. Numerous studies show that obesity is an independent predictor of cardiovascular disease as well as CVD risk factors including hypertension and diabetes, dyslipidemia (3-6). The most worrisome is increasing incidence and prevalence of diabetes, 60% of which can be contributed directly to weight gain (7). The health care cost of obesity is also quite staggering and by some estimates approaching 75 billion dollars per year (8).

I.A. Role of visceral fat

Obesity is characterized by an excess of adipose tissue. However, distribution of adipose tissue is not homogenous. Rather, it can be divided into different compartments. Jean Vague in 1946 first suggested that lower body fat (gynoid body habitus) had minor effect on health while upper body fat (android body habitus) was associated with diabetes and atherosclerosis (9). More recently, intraabdominal adipose tissue or visceral fat has emerged as the clinically relevant type of fat independent of total body fat (10-12). Numerous studies have suggested that central obesity or adipose tissue accumulation in central trunk area has greater cardiovascular adverse effect than adipose tissue deposition in peripheral areas (11,13,14). This suggests that someone with a normal BMI can still be at increased risk for cardiovascular complications if the visceral fat or abdominal obesity is increased (11). Even more worrisome is a combination of both an elevated BMI and visceral fat which can place an individual at increased risk for metabolic syndrome, diabetes, and cardiovascular disease. Many studies have shown that abdominal obesity is associated with increase in risk of coronary artery disease and mortality (15-18). It has also been reported that central adiposity has more detrimental effects on blood pressure than peripheral fat accumulation (19,20). Studies report that excess visceral fat is associated with high plasma TG and lower HDL levels (21-23).

Body fat distribution also differs between genders and varies with age and ethnicity. Visceral fat is noted to increase substantially with aging in females (24-26). Typically, obese black women have less visceral fat than obese white women at similar BMIs and WHRs (27,28). Despite having less visceral fat, blacks were noted to be at higher risk for type 2 diabetes than whites (28,29). Thus, it is possible that in obese black females, accumulation of visceral fat is even more ominous than other ethnicities. If this is indeed true, it may help explain the reason behind higher cardiovascular mortality among black females.

Abdominal obesity can be easily determined by measuring waist circumference, the waist-to-hip circumference ratio (WHR), or the less-commonly used sagittal abdominal diameter. Because of the methodologic difficulties in measuring abdominal fat compartments, waist circumference was recommended as a surrogate marker for visceral fat (30,31) and is considered to be preferable to the waist-to-hip ratio for this purpose (32). However, sophisticated imaging techniques such as magnetic resonance imaging (MRI), computed tomography (CT), ultrasound (US) and Dual Energy X-ray Absorbometry (DEXA) exist today making it possible to accurately differentiate fat depots at the waist level and measure visceral fat with high fidelity.

II. Pulse pressure and its role in development of cardiovascular disease

Pulse pressure (PP) is an independent cardiovascular risk factor (33). Elevated pulse pressure increases cardiovascular morbidity and mortality because of an elevation of systolic blood pressure (SBP), which raises left ventricular afterload, and because of a decrease in diastolic blood pressure (DBP), which alters coronary perfusion (34) (35). Franklin et al demonstrated that the prognostic effect of brachial PP on coronary heart disease was as strong as that of systolic BP (36-38) . Pulse pressure is also considered to be a surrogate measure of aortic stiffness (39). Increased aortic stiffness is a marker for increased cardiovascular morbidity as well as mortality (40,41) in multiple subgroups including diabetics, hypertensive, elderly, and patients with kidney disease (42). Thus, PP is an important intermediary phenotype to examine with regards to its link to visceral obesity.

II.A. Aortic stiffness and Pulse Pressure

Pulse pressure increases with age, largely due to increased systolic blood pressure and decreased diastolic pressure and thought to be due to increase in arterial stiffness. Arterial stiffness increases afterload (43) and myocardial oxygen demand (44-46), impairs ventricular relaxation(46) , and causes subendocardial ischemia (47). Increased pulse pressure is shown to be an independent predictor of risk of congestive heart failure in elderly cohort (48) which is a leading cause of hospitalization and accounts for significant economic burden of \$5 billions/yr. Thus, development of

increased pulse pressure has a tremendous impact on cardiovascular morbidity and mortality and contributes significantly to the total health care cost.

III. Association of Visceral fat and Pulse Pressure/aortic stiffness

There are limited data suggesting an association between obesity and aortic stiffness (49-51). The first study that investigated the effect of obesity on the mechanical properties of large arteries showed that Pulse wave velocity of the upper limbs was increased in obese, compared to non-obese, individuals (52). More recently, Robinson et al showed increased stiffness in the proximal descending aorta in an obese population without other underlying risk factor such as hypertension, diabetes, insulin resistance or hypercholesterolemia (53). Interestingly, central obesity is more strongly associated with increased aortic stiffness than increased BMI (41,54,55).

III.A Role of inflammatory markers and adipokines

Evidence exists that adiposity related increase in adipokines and inflammation may contribute to endothelial dysfunction. Clinical studies have also observed an association between elevated CRP level and increased aortic stiffness (56-59). However, it remains unclear whether inflammation leads to the development of aortic stiffness or whether increased in aortic stiffness results in inflammation as suggested by Abramson et al (60). The cellular mechanisms linking obesity and aortic stiffness and pulse pressure are complex. It is known that traditional risk factors and insulin resistance are largely responsible for endothelial dysfunction and CVD risk. However, emerging

research suggests that molecular links between obesity and endothelial dysfunction may be through the effects of fat-derived adipokines on endothelial function and vascular health (61-63). Mature adipocytes act as an active endocrine and paracrine organ. Adipose tissue is considered a rich source of proinflammatory mediators that may directly contribute to vascular injury, insulin resistance, and atherogenesis.

These proinflammatory adipocytokines, or adipokines, include TNF- α , IL-6, leptin, plasminogen activator inhibitor-1 (PAI-1), angiotensinogen, resistin, and, more recently, C-reactive protein (CRP) among others. Besides these deleterious adipokines, adipocytes release NO (64) and adiponectin which confer protection against inflammation and obesity-linked insulin resistance (65). Visceral fat produces several of these adipokines in much greater amounts than other fat depots (66) making visceral fat an important contributor to development of insulin resistance, endothelial dysfunction and ultimately cardiovascular disease. Much of the proinflammatory properties of adipocytes are through upregulation of CRP synthesis by circulating adipokines. CRP is an acute-phase reactant synthesized mainly in the liver and is regulated by circulating levels of IL-6, although TNF- α can also induce hepatic CRP mRNA expression (67). Adipose tissue derived IL-6 appears to be a major regulator of hepatic CRP production (68). Thus the amount of adipose- derived IL-6 is likely much higher in obese subjects who have both an increased total body fat mass and adipokine overexpression (69). CRP levels are directly correlated with high waist circumference, BMI, and with metabolic syndrome (70,71). CRP directly participates in the process of atherogenesis by modulating

endothelial function (72,73). CRP also induces the expression of VCAM-1, ICAM-1, selectins, and MCP-1 in cultured endothelial cells via increased secretion of ET-1, a potent endogenous vasoconstrictor, and IL-6 (72,73). CRP attenuates basal and stimulated endothelial NO production by downregulating endothelial NO synthase mRNA and protein expression (112) (74). CRP also plays a coordinating role by amplifying the proinflammatory activity of other adipokines. For example, it increases the expression and activity of PAI-1 in endothelial cells (75) which suppresses fibrinolysis by inhibiting plasminogen activation promoting thrombus formation. Recently, Lau et al reported that CRP expression in rat and mouse as well as human adipose tissue in both mature and developing fat cells suggesting that adipose tissue may be an independent source of plasma CRP.

TNF- α is another key inflammatory cytokine which is released in greater quantities by obese humans. It not only initiates, but also propagates atherosclerotic lesion formation by activation of the transcription factor nuclear factor- κ B (NF- κ B) and inducing the expression of VCAM-1, ICAM-1, MCP-1, and E-selectin in aortic endothelial and vascular smooth muscle cells (76). TNF- α also reduces NO bioavailability in endothelial cells and impairs endothelium-dependent vasodilatation, promoting endothelial dysfunction (77).

There is some evidence that weight loss is associated with a decrease in the serum levels of most of these adipokines, with the exception of adiponectin, which is increased (66,78). Animal studies have shown that surgical removal of visceral fat results in marked decrease in the gene expression of tumor necrosis factor and leptin (79). Further, emerging data suggest the beneficial effects of medications such as TZDs, statins and angiotensin-converting enzyme inhibitors in reducing the level of adipokines and subsequently endothelial dysfunction and atherosclerosis (80). Thus, it is hypothesized that CRP, IL-6 and TNF- α may be the key inflammatory markers in the development of endothelial dysfunction.

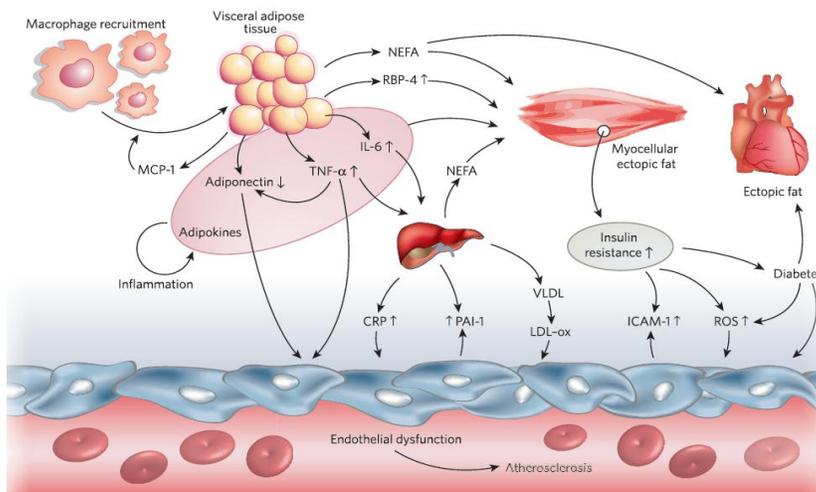


Fig 1. A schematic presentation of mechanism linking visceral adipose tissue to endothelial dysfunction. Printed with permission. Van Gaal et al (81).

Free fatty acid (FFA) drainage hypothesis once thought to be the main mechanism linking visceral adipose tissue to metabolic syndrome and endothelial dysfunction. Visceral adipose tissue produces large quantity of non-esterified free fatty

acid and delivers it to the liver with its easy access to portal circulation. The delivery of FFA to the liver results in synthesis of triglyceride (TG) rich very low density lipoproteins (VLDLs) which subsequently result in synthesis of TG rich LDLs. These TG rich LDLs are highly atherogenic contributing to endothelial dysfunction. Furthermore, FFA production by visceral adipocytes induces macrophages to produce TNF- α which causes production of IL-6 by adipocytes which in turn increases production of CRP by liver. High density lipoprotein (HDL) numbers are also decreased by hepatic lipases which lead to synthesis of smaller, denser HDL particles that are less effective at quenching reactive oxygen species. Smaller HDL particles are also easily eliminated from circulation further causing decrease in HDL.

Another mechanism that is proposed to link visceral adipose tissue to endothelial dysfunction and hypertension is through production of angiotensinogen by visceral adipocytes. In Framingham offspring study, 65-78% of hypertension was attributed to obesity (82). Adipose tissue is noted to be a producer of angiotensinogen (83,84). Angiotensinogen is converted to angiotensin I and subsequently to angiotensin II. This causes increase in sodium reabsorption in renal tubular cells resulting in arterial hypertension. Angiotensin II is also noted to hamper the maturation of pre-adipocytes to adipocytes resulting in large dysfunction adipocytes which in turn produce more angiotensinogen. This relationship is supported by study where 5% weight loss resulted in decrease in ambulatory blood pressure as well as decrease in angiotensinogen, renin and angiotensin converting enzyme activities (85). Aldosterone has been implicated in

obesity mediated hypertension as obese individual have higher plasma aldosterone level, however, exact mechanism is unclear. Further, activation of sympathetic nervous system has been proposed as a mechanism linking visceral obesity to hypertension and subsequent aortic stiffness. Adiponectin suppresses the sympathetic nervous system which is instrumental in development of hypertension. Visceral adipocytes decrease the production of adiponectin in turn causing increase in sympathetic nervous system resulting in hypertension. Further, local compression of renal lymphatics by visceral adipose tissue can activate renin-angiotensin-aldosterone system promoting hypertension, endothelial dysfunction and atherosclerosis.

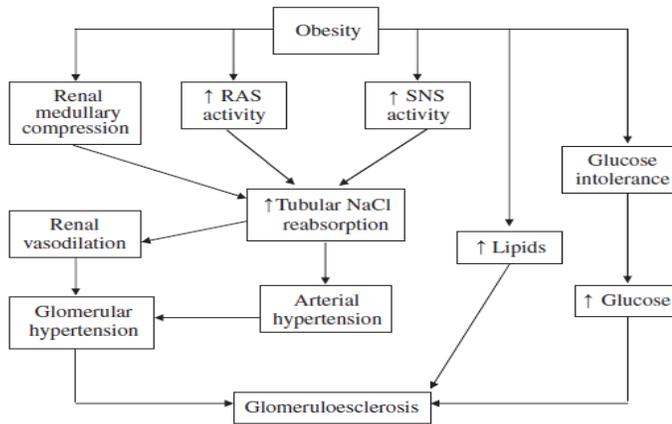


Fig 2. Obesity mediated hypertension: A proposed mechanism. Adapted from Segura et al (86).

IV. Summary

In summary, obesity, and notably abdominal obesity, increases CVD risk through its effects on insulin resistance and endothelial function. Pulse pressure is considered a

cardiovascular risk factor. Increased pulse pressure is shown to be a strong predictor of general and cardiovascular mortality, especially coronary mortality. Pulse pressure is also considered to be a surrogate measure of aortic stiffness. Increased aortic stiffness has been shown to be a marker for increased cardiovascular morbidity as well as mortality in multiple subgroups including diabetics, hypertensive, elderly, and patients with kidney disease. Visceral obesity is associated with worsening in aortic stiffness leading to adverse cardiovascular outcomes. Evidence is mounting to suggest a more direct role for adipokines in endothelial and cardiovascular health and that these effects are independent of their influence on insulin resistance and diabetes.

V. Purpose

The purpose of this study proposal is to examine the association between visceral fat and pulse pressure (as a surrogate for aortic stiffness) in the MESA participants. We will further explore the role of ethnicity and gender on this association by stratifying our data by ethnicity and gender. This information may be useful in determining the cause of discrepancy in cardiovascular morbidity and mortality among Caucasian and African-Americans. It may further help target therapies for prevention or treatment of cardiovascular disease, or for better risk stratification of individuals, which may impact the leading cause of mortality worldwide.

Main Objective

To define the association between central obesity as measured by visceral fat using abdominal CT and pulse pressure in the MESA cohort.

Research Hypothesis:

1 – Visceral fat is associated with pulse pressure in the MESA cohort.

DATA

Sample: 398 MESA cohort members from Forsyth County, NC who already have an abdominal CT examination during exam 2 or 3 as a part of ancillary study by Dr. Jingzhong Ding.

Dependent Variable:

Pulse pressure:

Pulse pressure = Systolic blood pressure – Diastolic blood pressure

Independent Variables:

CT derived measure of visceral fat (cm²)

Co-variates:

Demographic: Age, gender, ethnicity

History: Type 2 diabetes mellitus

Medications: use of hypertensive medication (variable)

Anthropometrics: heart rate, BMI.

ANALYSIS PLAN AND METHODS:

In analyzing the relationship between visceral fat and aortic stiffness, we will use linear regression. We will treat visceral fat as the independent variable and will approach pulse pressure as the dependent variable. We will use both variables as continuous variable. Adjustments for confounding variables will be performed.

SIGNIFICANCE:

If we find an association between visceral fat and pulse pressure (as a surrogate for aortic stiffness), we can postulate that changes in pulse pressure may be mediating the association between body composition and cardiovascular risk. Further, we can identify ethnic differences in this association which may provide novel clues to the pathogenesis of cardiovascular disease in ethnic minorities, and may help in further risk stratification in this population.

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CHAPTER 2

Associations between Visceral Adipose Tissue and Pulse Pressure in the Multi-Ethnic Study of Atherosclerosis (MESA)

Jain M¹, Ding J¹, Allison M², Criqui M², Carr J¹, and Herrington DM¹

¹Section on Cardiology, Wake Forest University School of Medicine; Winston-Salem, NC

² University of California, San Diego, CA

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Abstract

Background: Obesity and aortic stiffness (AS) contribute to cardiovascular disease (CVD). Central obesity as measured by Visceral Adipose Tissue (VAT) is thought to be a more specific measure of deleterious adiposity independent of body mass index (BMI). However, the nature of the relationship between VAT and AS in different sex and ethnic-specific subgroups is not well established.

Methods: The association between VAT and PP was assessed in a subset of MESA - a cohort of subjects aged 45-84 yrs without clinical CVD. VAT (mm^3) was estimated using 5, 3mm abdominal computed tomography slices centered at the L4–L5 level. PP (systolic– diastolic BP) - a surrogate for AS, and VAT variable were log transformed to reduce skewness. Multivariable linear regression models were used to describe the relationship between PP and VAT with and without adjustment for age, gender, heart rate, race, diabetes, and use of hypertensive medication.

Results: VAT and PP data were available for 398 subjects (mean age 65 yrs, 53% female, 57% Whites, 43% African-American (AA), mean Log VAT 2.23 ± 0.20 , mean Log PP 1.74 ± 0.13 mmHg). In univariate analysis, VAT was significantly associated with PP ($p = 0.0038$). Exploratory analysis revealed a significant interaction with gender ($p < 0.001$), with stronger associations in women than in men. In multivariate adjusted analyses stratified by gender there was a significant relationship between VAT and PP in women

($p = 0.0004$) but not in men ($p = 0.24$). This gender-specific relationship was stronger in African American women ($p = 0.0003$), than white women ($p = 0.02$).

Conclusions: Overall, VAT was associated with PP in this small sample of the MESA cohort. However, subgroup analysis revealed that the relationship between VAT and PP is modified by gender. This association was particularly strong in AA women. These data suggest that in AA women, VAT may have higher significance despite observations that AA women have lower VAT than other ethnicities. These data illustrate the need for more extensive and ethnic- and gender-specific research on the potential significance of VAT and its role in the pathophysiology of CVD.

Introduction

Obesity is a global public health challenge. Numerous studies show that obesity is an independent predictor of cardiovascular disease as well as CVD risk factors including hypertension and diabetes, dyslipidemia (3-5). More recently, intraabdominal adipose tissue or visceral fat has emerged as the clinically relevant type of fat independent of total body fat (10). Numerous studies have suggested that central obesity or adipose tissue accumulation in central trunk area has greater cardiovascular adverse effect than adipose tissue deposition in peripheral areas (13,14). Central adiposity has more detrimental effects on blood pressure than peripheral fat accumulation (19), and is associated with high plasma TG and lower HDL levels (21-23).

This distribution in body fat differs with ethnicity and gender. African-American women have the highest prevalences of obesity (2) and obese black women have less visceral fat than obese white women at similar body mass index and waist to height ratio (27,28). Despite having less visceral fat, blacks were noted to be at higher risk for type 2 diabetes than whites (28,29).

Pulse pressure (PP) is an independent cardiovascular risk factor (33). Franklin et al demonstrated that the prognostic effect of brachial PP on coronary heart disease was as strong as that of systolic BP (36-38) . Pulse pressure is also considered to be a surrogate measure of aortic stiffness (39). Increased aortic stiffness is a marker for increased cardiovascular morbidity as well as mortality (40,41) in multiple subgroups including diabetics, hypertensive, elderly, and patients with kidney disease (42).

Relationship between visceral adipose tissue (VAT) and pulse pressure has not been investigated. Thus, the purpose of the present study is to examine the relationship between CT measures of VAT and pulse pressure as well as to assess the gender and ethnic difference in this relationship.

Methods

Study Sample

The Multi-Ethnic Study of Atherosclerosis (MESA) is a community-based cohort study designed primarily to investigate the prevalence, correlates, and progression of

subclinical cardiovascular disease (17). A total of 6814 whites, blacks, hispanics, and Asian Americans aged 45–84 y were recruited from Baltimore, MD, Chicago, IL, Forsyth County, NC, Los Angeles, CA, New York, NY, and St Paul, MN, in 2000–2002. Individuals with physician-diagnosed cardiovascular disease were not eligible. The study was approved by the Institutional Review Boards of the participating institutions, and the participants gave informed consent. All MESA participants underwent collection of anthropometry and cardiovascular risk factor data at either visit 2 or 3. A random sample of 398 MESA white and black participants (47% men, 43% black), aged 47–86 y, in Forsyth County, NC, also received abdominal CT scans as a part of ancillary study. One of the measurements collected on these abdominal CTs was volume of visceral adipose tissue.

Fat depots

Abdominal visceral and subcutaneous adipose tissue volumes were measured on abdominal CT scans. Technical factors for abdominal CT scans were helical mode, 120 kVp, 250 mA, 4-2.5 mm collimation, standard reconstruction kernel, and a display field of view of 500 mm. For abdominal visceral, and subcutaneous fat volumes, slices within 15 mm centered at the L4–L5 level were selected. We manually traced the inner and outer aspects of the abdominal wall. Abdominal visceral fat was defined as the fat enclosed by the inner aspect of the abdominal wall. Abdominal subcutaneous fat was defined as the fat outside the outer aspects of the abdominal wall. Studies of human cadavers showed that the area measured by CT scanning was an accurate estimate of

abdominal visceral fat (4), appendicular subcutaneous and intermuscular fat (6) volumes. To examine the reproducibility of the measures of fat depots, a random sample of 80 MESA participants was selected, and their CT scans were reanalyzed masked to the prior results. The intraclass correlation coefficients of intrareader and interreader reliability were 0.99 and 0.99 for abdominal visceral, subcutaneous, and intermuscular fat.

Anthropometry

Weight was measured with a Detecto Platform Balance Scale (Detecto, Webb City, MO) to the nearest 0.5 kg. Height was measured with a stadiometer [Accu-Hite Measure Device (Seca, Hamburg, Germany) with level bubble] to the nearest 0.1 cm. Waist circumference (at the umbilicus) was measured to the nearest 0.1 cm with the use of a steel measuring tape with standard 4-ounce tension (Gulick II 150 cm anthropometric tape; Sammons Preston, Chicago, IL). Body mass index (in kg/m²) was calculated.

Other covariates

Standardized questionnaires were used to collect information on demographics, smoking status, alcohol use, medical history, and medication use. Cigarette smoking status was classified as never, former, and current. Blood pressure was measured in the right arm of the participant after 5 min in a sitting position with a Dinamap model Pro 100 automated oscillometric sphygmomanometer (Critikon, Tampa, FL). The second and

third of 3 readings were averaged to obtain the blood pressure values. HDL cholesterol and triacylglycerols were measured in EDTA-treated plasma on a Roche COBAS FARA centrifugal analyzer (Roche Diagnostics, Indianapolis, IN). Glucose was measured by rate reflectance spectrophotometry with the use of thin film adaptation of the glucose oxidase method on the Vitros analyzer (Johnson & Johnson Clinical Diagnostics Inc, Rochester, NY). Diabetes was defined as fasting glucose >6.99 mmol/L (126 mg/dL) or the use of hypoglycemic medication, and impaired fasting glucose was defined as fasting glucose 5.55– 6.94 mmol/L (100–125 mg/dL) (22).

Statistical analysis

The distributions of VAT and pulse pressure were not normal. Therefore, the pulse pressure and VAT distribution were normalized through a natural log transformation. Means and standard deviations or proportions were calculated for selected variables. We first evaluated association of natural log of pulse pressure with selected variables using Pearson correlation coefficients. For the primary analysis, multiple linear regression models were used to examine the association between pulse pressure and VAT. Through a stepwise approach, we included selected variables which were noted to be significant in univariate analyses including age, heart rate, gender and race. Interactions with gender and race were tested for VAT. The final model included race and gender subgroups to determine whether associations differed by race and gender. We finally added BMI to our model to ascertain whether visceral adipose tissue

predicts pulse pressure beyond BMI. The presentation of the multivariate model included the standardized coefficients.

To evaluate how central adiposity is associated in the entire MESA population particularly in Caucasian and African-American populations, we perform the above analysis using waist circumference as a measure of central adiposity. We used pulse pressure as our dependent variable and waist circumference as an independent variable. Using multiple linear regression, we assessed the relationship between waist circumference and pulse pressure after adjusting for variables including age, use of hypertensive meds, cholesterol, diabetes and heart rate. SAS version 9.00 (SAS Institute Inc, Cary, NC) was used for the analysis.

Results

The visceral adipose tissue and pulse pressure data was available for 398 participants. The mean age of these participants was 65 yrs; 53% were female, and 43% were African-American. The mean Log visceral adipose tissue was 2.23 mm³ with standard deviation of 0.20; mean Log pulse pressure was 1.74 mmHg with standard deviation of 0.13. The clinical characteristics of participants are presented in Table 1.

In univariate analysis, age was most strongly associated with pulse pressure followed by gender and anti-hypertensive medication (Table 2). Other factors that were associated with pulse pressure were visceral adipose tissue and higher blood lipid levels. A significant negative association was observed between heart rate and pulse pressure.

Interestingly, diabetes and race were not significantly associated with pulse pressure in our analysis.

In multivariate analysis, visceral adipose tissue remains independently associated with pulse pressure ($p=0.001$) even after stepwise addition of age, gender, heart rate, cholesterol, diabetes and use of anti-hypertensive medication in our model (Table 3). Other factors that were significantly associated with pulse pressure were age, heart rate, hypertensive medication use and gender. Again, race and diabetes were not predictive of pulse pressure in our analysis. In multivariate adjusted analyses stratified by gender showed that there was a significant relationship between viscera adipose tissue and pulse pressure in females ($p=0.0004$) but not in males ($p=0.24$) (Table 4). We then investigated whether the association between visceral adipose tissue and pulse pressure differs among race; and further stratified our model by both race and gender. We found that this gender-specific relationship was stronger in African-American females (Std β Coefficient 3.78, $p=0.0003$) than Caucasian females (Std β Coefficient 2.31, $p=0.02$) (Table 5). To determine whether there is a significant interaction between visceral adipose tissue, gender or race, we added race and gender as interaction terms into our model. This analysis revealed a significant interaction with gender ($p<0.001$), while no interaction was noted with race. Finally, we also included BMI in the model to further ascertain whether visceral adipose tissue is an independent predictor of pulse pressure beyond BMI. This analysis revealed that in African-American females, visceral adipose tissue is an independent predictor of pulse pressure even after adjusting for BMI (Std β

Coefficient 2.48, $p=0.015$) while no significant relationship was found in Caucasian females ($p=0.43$), African-American males or Caucasian males.

We also performed the similar analysis in Caucasian and African-American populations of entire MESA cohort using waist circumference as a measure of central adiposity. We found that waist circumference was significantly associated in both male and female and there were no ethnic differences between the two ethnicity (Table 7). However, after addition of BMI to the model, waist circumference was significantly associated with pulse pressure only in Caucasian males (Std β coefficient 2.66, P value 0.008, data not shown).

Discussion

In this study, the main finding is that visceral adipose tissue is not a significant predictor of pulse pressure in males. In females, however, visceral adipose tissue remains a significant predictor of pulse pressure after adjustment for known predictor of pulse pressure including age, heart rate, diabetes, use of hypertensive medication and race. This association between visceral adipose tissue and pulse pressure seems to vary with ethnicity. In African-American females, visceral adipose tissue appears to be a stronger predictor of pulse pressure than Caucasian females. This association remains significant in African-American females even after adjusting for BMI.

Although visceral adipose tissue is shown to increase substantially with aging in females (24), our study also noted the ethnic difference in this association. The gender

difference in association of visceral adipose tissue with systolic blood pressure and diastolic blood pressure has been previously reported in Framingham Heart Study cohort (87). In NHANES III cohort, BMI was also strongly associated with pulse pressure (88,89). In our study, we found that the association between visceral adipose tissue and pulse pressure is stronger among African-American than Caucasian females. Further, visceral adipose tissue remained a predictor of pulse pressure independent of BMI in African-American females. This finding is particularly intriguing since African-American females are shown to have less visceral adipose tissue when compared to Caucasians. Despite having lower visceral adipose tissue, African-Americans have higher cardiovascular morbidity and mortality, the reason for which remains unclear. This data suggests that visceral adipose tissue in African-American females may have more deleterious cardiovascular effect than their Caucasian counterparts which may be contributing to the higher prevalence and risk of cardiovascular morbidity and mortality in African-Americans.

When we used waist circumference as a measure of central adiposity in a larger sample size using MESA cohort, we did not see the above mentioned gender and ethnic difference. In Caucasian males, waist circumference remains a significant predictor of pulse pressure independent of BMI. These results may be due to the fact that waist circumference is not a true measure of central adiposity and thus may not accurately reflect the relationship between visceral adipose tissue and pulse pressure. Another drawback of using waist circumference is poor reproducibility of this measure which may

have contributed to the discrepancy in our results. Of course, one would have to consider the possibility that we may have seen the association between VAT and pulse pressure due to chance especially given our small sample size. However, we know that VAT is a much more specific measure of central adiposity. Further, our results are consistent with prior studies that women have higher VAT, and gender difference exists in relationship between VAT and cardiovascular disease risk markers. Larger studies are needed to conclusively answer this question.

The cellular mechanisms linking obesity and aortic stiffness are complex. It is known that traditional risk factors and insulin resistance are largely responsible for endothelial dysfunction and CVD risk. However, emerging research suggests that molecular links between obesity and endothelial dysfunction may be through the effects of fat-derived adipokines on endothelial function and vascular health. Mature adipocytes act as an active endocrine and paracrine organ. Adipose tissue is considered a rich source of proinflammatory mediators that may directly contribute to vascular injury, insulin resistance, and atherogenesis. These proinflammatory adipocytokines, or adipokines, include TNF- α , IL-6, leptin, plasminogen activator inhibitor-1 (PAI-1), angiotensinogen, and resistin. Besides these deleterious adipokines, adipocytes release NO (64) and adiponectin which confer protection against inflammation and obesity-linked insulin resistance (65). Visceral adipose tissue produces several of these adipokines in much greater amounts than other fat depots (66) making visceral adipose tissue an important contributor to development of insulin resistance, endothelial dysfunction and ultimately

cardiovascular disease . Visceral adipose tissue can also leads to insulin resistance and likely causes endothelial dysfunction by both elevated insulin and glucose levels.

Our study does have several limitations. Our sample size is fairly small. Our study may not be sufficiently powered to detect the association between visceral adipose tissue and pulse pressure in men. However, the fact that we were able to see a significant association among females with a small sample is reassuring. Further, because of its cross-sectional study design, the results of this study should be interpreted cautiously that this association does not prove causality. These data illustrate the need for more extensive and ethnic and gender-specific research on the potential significance of visceral adipose tissue and its role in the pathophysiology of cardiovascular disease.

Table 1. Baseline Characteristics of MESA participants

| Variables | |
|--|-------------------|
| Age (yrs) | 64.5 ± 9.6 |
| Caucasian (%) | 57 |
| African-American (%) | 43 |
| Female (%) | 53 |
| Systolic Blood Pressure (mmHg) | 128 ± 21 |
| Diastolic Blood Pressure (mmHg) | 70.4 ± 9.7 |
| Heart Rate (bpm) | 64 ± 10 |
| Cholesterol | 184 ± 36 |
| BMI (Kg/m²) | 29 ± 5.2 |
| Hypertension medication use (%) | 47 |
| Diabetes (%) | 13 |

Value expressed as mean ± standard deviation or percentage. BMI=Body Mass Index

Table 2. Unadjusted associations with Log of Pulse Pressure

| Variables | Std β Coefficient | p Value |
|------------------------------------|---|--------------------|
| Age | 8.82 | < 0.0001 |
| Gender | 5.50 | <0.0001 |
| HTN meds | 4.89 | <0.0001 |
| Log Visceral adipose tissue | 2.91 | 0.0038 |
| Heart rate | -2.88 | 0.0043 |
| Cholesterol | 2.14 | 0.0328 |
| Race | 1.32 | 0.1876 |
| Diabetes | 1.56 | 0.1176 |

STD= Standard, HTN= Hypertension, Meds = Medications

Table 3. Independent adjusted associations with Log of Pulse Pressure

| Variables | Std β Coefficient | p Value |
|------------------------------------|---|--------------------|
| Age | 8.13 | < 0.0001 |
| Gender | 5.69 | <0.0001 |
| HTN meds | 3.25 | 0.0013 |
| Log Visceral adipose tissue | 3.35 | 0.0010 |
| Heart rate | -2.30 | 0.0221 |
| Cholesterol | 3.46 | 0.0006 |
| Race | 1.28 | 0.2010 |
| Diabetes | 0.33 | 0.7380 |

STD= Standard, HTN= Hypertension, Meds = Medications

Table 4. Independent adjusted associations with Log of Pulse Pressure when data is stratified by gender

| Variables | Male (n=188) | | Female (n=210) | |
|-----------------------------|-------------------------|---------|-------------------------|---------|
| | Std β Coefficient | p Value | Std β Coefficient | p Value |
| Log Visceral adipose tissue | 1.17 | 0.2420 | 3.62 | 0.0004 |
| Age | 5.45 | < .0001 | 6.17 | < .0001 |
| Heart rate | -2.28 | 0.0238 | -0.98 | 0.3300 |
| Cholesterol | 2.12 | 0.0347 | 2.80 | 0.0049 |
| HTN meds | 3.32 | 0.0010 | 1.18 | 0.2400 |
| Race | 0.93 | 0.3519 | 0.76 | 0.4509 |
| Diabetes | 0.09 | 0.9248 | -0.57 | 0.5670 |

Cau = Caucasian, AA = African-American, STD= Standard, HTN= Hypertension, Meds = Medications

Table 5. Independent adjusted associations with Log of Pulse Pressure in females

| Variables | CAU female (n=113) | | AA female (n=97) | |
|-----------------------------|-------------------------|---------|-------------------------|---------|
| | Std β Coefficient | p Value | Std β Coefficient | p Value |
| Log Visceral adipose tissue | 2.31 | 0.0225 | 3.78 | 0.0003 |
| Age | 5.27 | < .0001 | 2.99 | 0.0038 |
| Heart rate | 0.44 | 0.6629 | -1.92 | 0.0579 |
| Cholesterol | 1.79 | 0.0757 | 2.56 | 0.0118 |
| HTN meds | 0.11 | 0.9158 | 2.39 | 0.0188 |
| Diabetes | 1.07 | 0.2845 | 0.84 | 0.4049 |

Cau = Caucasian, AA = African-American, STD= Standard, HTN= Hypertension, Meds = Medications

Table 6. Independent associations with Log of Pulse Pressure in females adjusted for BMI

| Variables | CAU female (n=113) | | AA female (n=97) | |
|-----------------------------|-------------------------|---------|-------------------------|---------|
| | Std β Coefficient | p Value | Std β Coefficient | p Value |
| Log Visceral adipose tissue | 0.79 | 0.4310 | 2.48 | 0.0148 |
| Age | 5.42 | < .0001 | 3.17 | 0.0018 |
| Heart rate | 0.51 | 0.6136 | -1.94 | 0.0562 |
| Cholesterol | 1.66 | 0.1001 | 2.41 | 0.0183 |
| HTN meds | 0.12 | 0.9081 | 2.25 | 0.0274 |
| Diabetes | 1.20 | 0.2324 | 0.97 | 0.3351 |
| BMI | 1.23 | 0.2231 | 1.04 | 0.3004 |

Cau = Caucasian, AA = African-American, STD= Standard, HTN= Hypertension, Meds = Medications, BMI = Body mass index

Table 7. Multivariate associations with Pulse Pressure in MESA cohort when data is stratified by gender and race.

| | Male | | Female | |
|-------------|-----------------------------------|----------------|-----------------------------------|----------------|
| | Std β Coefficient (p value) | | Std β Coefficient (p value) | |
| | CAU (N=837) | AA (N= 657) | CAU (N=841) | AA (N=744) |
| Waist Circ. | 4.38 (<0.001) | 4.90 (<0.001) | 4.41 (<0.001) | 3.53 (0.001) |
| Age | 11.80 (<0.001) | 9.17 (<0.001) | 16.61 (<0.001) | 9.93 (<0.001) |
| Heart Rate | -2.95 (0.003) | -5.32 (<0.001) | - 3.31 (0.001) | -5.66 (<0.001) |
| Cholestrol | 2.23 (0.026) | 0.99 (0.322) | 1.29 (0.197) | 2.96 (0.003) |
| HTN meds | 1.70 (0.089) | 2.34 (0.020) | 5.11 (<0.001) | 4.02 (<0.001) |
| Diabetes | 4.91 (<0.001) | 3.18 (0.002) | 1.71 (<0.001) | 4.54 (<0.001) |

Cau = Caucasian, AA = African-American, STD = Standard, Circ = Circumference,
Meds = Medications, HTN = Hypertension

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CHAPTER 3

Definition of Visceral Adipose Tissue (VAT) and Use of Computed Tomography (CT) in the measurement of Visceral Adipose Tissue in Multi-Ethnic Study of Atherosclerosis (MESA)

In Chapter 2, we identified the strength of association between abdominal visceral fat (VAT) and pulse pressure (PP) in Caucasian (CAU), and African American (AA) individuals in a subgroup of MESA. The third and final chapter of the thesis submitted sets out to discuss the definition of VAT and its measurement using CT. I will review the principles of CT imaging, its advantages and potential drawbacks.

I. Visceral Adipose Tissue (VAT)

Deposition of excess adipose tissue is the hallmark of obesity. It has been noted that adipose tissue is not a single homogenous compartment in the body. Rather, total adipose tissue in the body is a collection of regional deposition of adipose tissues which differ in cellular characteristics and biological function. Depending upon the location and composition of these depots, regional adipose tissues have variable physiological and pathological implication. There is not a formal consensus among experts in their definition of various compartments, largely due to adipose tissue composition and biologic function.

Although terms “fat” and “adipose tissue” are generally used interchangeably, some authors have made a distinction between the two. Adipose tissue is considered a loose connective tissue that is laden with adipocyte. Fat on the other hand, is lipid mainly in the form of triglyceride. Fat is found mainly in adipocytes but can also exist independently in other organs such as heart, liver and skeletal tissue, and has pathological implication. Adipose tissue can be readily quantified by various modalities while quantification of fat cells often requires spectroscopy. In the early days, adipose tissue has been classified based on its biological function and has been differentiated into white, brown, mammary gland and bone marrow adipose tissue. Other experts have organized into organ specific adipose tissue, subcutaneous tissue, and bone marrow tissue. One limitation of such classification is that it is extremely difficult to quantify adipose tissue using non-invasive techniques. For our discussion, I will focus on most recent classification of adipose tissue.

Generally speaking, adipose tissue can be divided into two major depot; subcutaneous adipose tissue and non-subcutaneous or internal adipose tissue (90).

Total adipose tissue = subcutaneous adipose tissue + internal adipose tissue

Subcutaneous adipose tissue has clear anatomical demarcation and is defined by layer found between dermis and the fascia of muscles. It can be further divided in superficial and deep subcutaneous adipose tissue; however, this terminology is seldom used in

clinical practice. Internal adipose tissue is further divided into visceral adipose tissue and non-visceral adipose tissue.

Internal adipose tissue= Visceral adipose tissue + non-visceral adipose tissue

Non-visceral adipose tissue is a generic term that is essentially a combination of difficult to quantify depots including intramuscular, intermuscular and para-osseal adipose tissue.

Nonvisceral adipose tissue= Intramuscular + Intermuscular + Paraosseal AT

Visceral adipose tissue is the main adipose tissue that is found in chest, abdomen and pelvic cavity. In the thoracic cavity, main deposit of visceral adipose tissue is found around the pericardium which has gained some interest over the last few years as potentially atherogenic and physiologically detrimental. In the abdominal cavity, visceral tissue has also been further divided into intraperitoneal and retroperitoneal adipose tissue. Some authors prefer this differentiation as these two depots of adipose tissue seem to have different metabolic activity (91). Others have gone even further to describe visceral adipose tissue specific to organs it surrounds, as in “perirenal adipose tissue.”

Abdominal visceral adipose tissue = Intraperitoneal AT + Retroperitoneal AT

In our discussion and calculation of visceral adipose tissue, we have used simplified and most commonly used approach to visceral tissue including both intraperitoneal and retroperitoneal visceral adipose tissue.

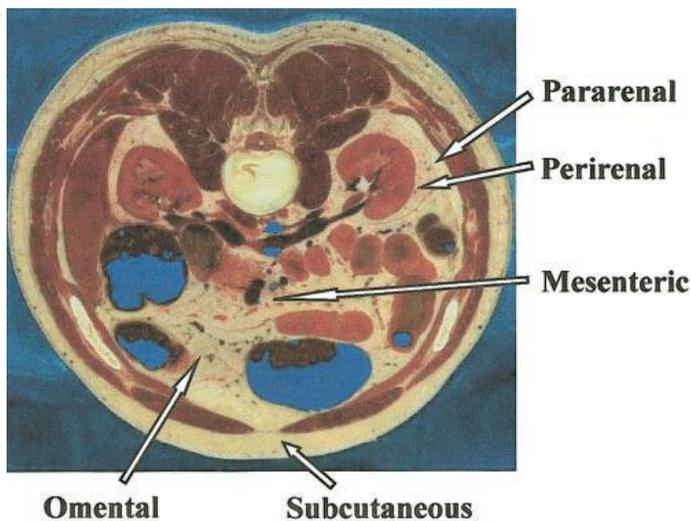


Figure 1: Adipose Tissue Compartment in an axial section. This figure shows different adipose tissue compartments in an axial section of abdominal region. Reprinted with permission from National Library of Medicine.

II. Quantification of VAT: Overview of available modalities

We have established that there is clear distinction between subcutaneous and visceral adipose tissue both in terms of their metabolic activity and their physiologic and clinical implication. For this reason, it is crucial to accurately identify and measure the visceral adipose tissue for purposes of research and clinical outcome.

The visceral adipose tissue can be quantified using various modalities that range from indirect estimation of visceral adipose tissue to sophisticated novel imaging modalities. I will briefly review these modalities, their advantages and disadvantages and then focus on computed tomography in depth.

II.A Anthropometry

VAT can be indirectly assessed by multiple anthropometric measurements. Body Mass Index (BMI), Waist Circumference (WC), hip circumference (HC) and Waist to hip Ratio (WHR) are most commonly used. BMI is likely the most commonly used measurement to assess obesity. It is highly reliable but it is not sensitive or specific for measurement of total body fat and VAT. Body weight comprises of muscle mass, adipose tissue and bone mass and this measurement does not differentiate between these compartments. Burke et al studied 59 males and directly measured visceral fat using MRI and indirectly using WC, WHR and BMI. Using multiple regression analysis, they noted that WC is the anthropometric index that uniformly predicts the distribution of adipose tissue among several fat depots in abdominal region including subcutaneous as well as visceral adipose tissue. However, they concluded that there is little utility in measuring WHR, or BMI in predicting VAT (92).

Waist circumference and Hip circumference are measurements that are easily obtainable; however, they are less reproducible. There is significant variation in WC depending upon the location of measurement. Typically, measurement is taken at the level of belly button, but some have used the midpoint between the lateral iliac crest and lowest rib. HC is usually measured as the widest circumference at the level of trochanteric major. These measurements especially WC are attractive in large population based studies due to their simplicity, low cost and somewhat acceptable accuracy.

Present literature shows that waist circumference is a sensible, but not specific, method for the identification of visceral fat in obese subjects. WHR and BMI have not been shown to be a reliable measure for evaluating visceral adipose tissue.

II.B Dual Energy X-ray Absorptiometry (DEXA)

DEXA scanning works on the principal that soft tissue and bone mineral have different attenuation under a constant X-ray source and varying X-ray energy level which is detected by a scanner. The measurement of fat is derived from the ratio of these measures which is recorded as R value. The R value is determined for all pixels that contain soft tissue but no significant bone. After a series of iterations, DEXA provides the in vivo fat mass, lean mass, and bone mineral content for the total body, and the arm, leg, and trunk regions. Fat in the abdominal region is distinguished by identifying the area as a specific region of interest (usually defined as upper edge of second lumbar

vertebra to the lower edge of fourth lumbar vertebra) within the analysis software as depicted in the figure below.

Several studies have evaluated the reliability of abdominal adipose tissue measurement and found it to be in good agreement with MRI (93), CT (94) and anthropometric measures (95). Intra-scanner variability is also excellent with $0.6 \pm 0.5\%$ variability in percent body fat (93). Thus, DEXA is reliable, quick, accurate and exposes individual to only small dose of ionizing radiation. However, it has several limitations. Most importantly, DEXA cannot distinguish intra-abdominal from subcutaneous fat which limits its utility in assessing the effect of visceral adipose tissue on cardiovascular health and though the ionizing radiation risk is low, the risk remains when compared to other modalities without any ionizing radiation.

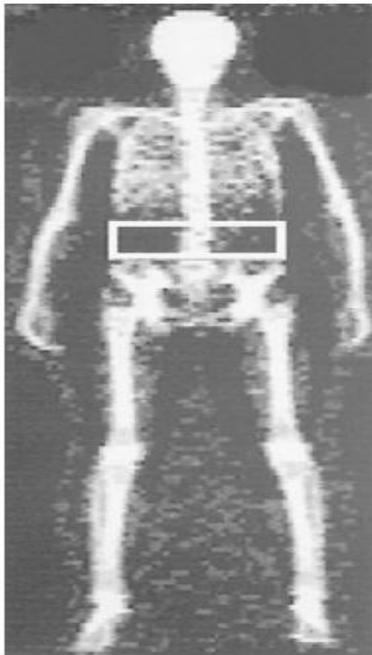


Figure 2. A representative image from a DEXA scan showing region of interest.

II.C Ultrasound

Ultrasound was first proposed as a tool to quantify visceral fat in 1990 by Armellini et al. as an alternative technique to CT (96). Ultrasound imaging is based on the principle that sound wave can travel through different mediums and partly reflect back after hitting the surface of a medium. This reflection depends on the difference in acoustic impedance of the two tissues. Subcutaneous fat, visceral fat and muscle tissue have different acoustic impedances which is used to measure visceral fat compartment. Subcutaneous fat is usually defined as the distance between the skin and external face of the rectus abdominis muscle, and visceral fat is defined as the distance between the internal face of the same muscle and the anterior wall of the aorta as shown in the figure below.

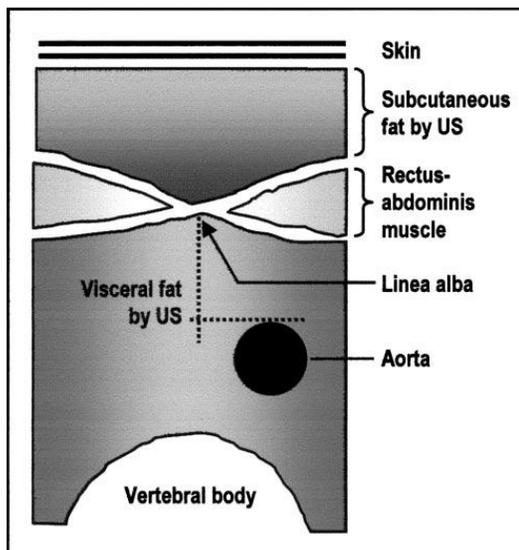


Figure 3 . Illustration of abdominal adipose tissue and anatomical landmark used for ultrasound measurements. Reprinted with permission. Armellini et al (96).

Ultrasound has shown good reproducibility with coefficient of variation of 1% (96-100), and acceptable correlations with other imaging modalities (101-103). It is readily available and inexpensive. Biggest limitation of ultrasound is that there are no specific cut offs to define visceral obesity and inter-observer variability is poor in clinical arena. It will likely require well trained sonographer and specific protocol for location of transducer, amount of pressure and timing of the measurement before the research derived inter-observer variability can be translated in clinical setting.

II.D Magnetic Resonance Imaging (MRI)

MRI is based on the principle that each atom in our body is consist of protons. In the presence of external magnetic field, these atoms possess magnetic properties themselves which can be quantified as T1 or T2 time. Depending upon the composition of the tissue, different tissues have different T1/T2 time in the presence of external magnetic field depending upon their relaxation time. The adipose tissue of various body compartments can be readily identified by MRI because fat has a different proton relaxation time as compared to other tissue constituents (Fig 4). Assuming that adipose tissue is composed of 84.67% fat, 12.67% water, and 2.66% proteins (104), the density of adipose tissue can calculated to be 0.9196 kg/l. Therefore, adipose tissue mass was calculated in kilograms for each 10-mm slice.

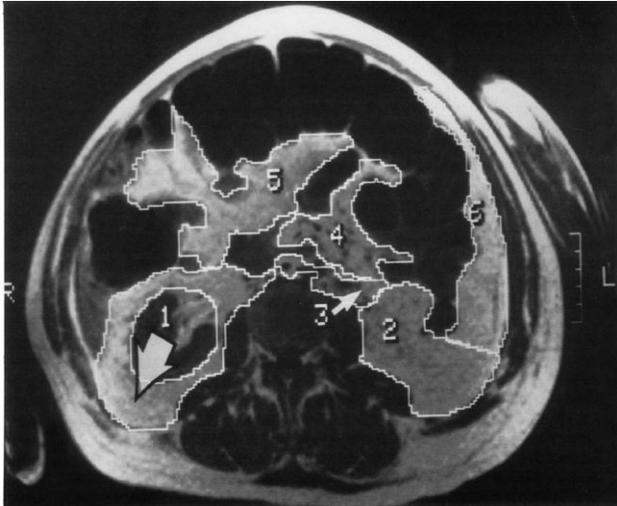


Figure 4. A representative MRI image of Intra-abdominal visceral tissue. Arrows points to visceral adipose tissue compartment. Section 1, 2, and 3 represents retroperitoneal compartment while 4, 5, and 6 represent intraperitoneal compartment. Reprinted with permission. Originally published in J Lipid Res. 1994; 35:1490-6. (91)

Initial study investigating the validity of MRI in estimating adipose tissue was done by abate et al (91) where they validated MRI estimated adipose tissue mass against direct weighing of the same adipose tissue compartments after dissection in human cadavers. The results showed that the mean of the difference between the two methods was only 0.076 kg (95% confidence interval: + 0.005 kg, + 0.147 kg). The coefficient of variation of visceral adipose tissue by MRI has also been well studied and ranges from 9% to 18% (105-108). Some authors have studied the feasibility of predicting visceral adipose tissue by using single MRI slice and concluded that single slice MRI at L2-L3 level can reliably and accurately predict the visceral adipose tissue (109).

MRI has several potential advantages including absence of radiation exposure, and its ability to determine the volume of a compartment. It is a highly precise method which is desirable for research. Despite these advantages, MRI has several disadvantages: MRI equipment is very expensive, requires trained technicians and is not readily available in clinical arena.

III. Computed Tomography (CT)

In the MESA, computed tomography was used as the modality to measure visceral fat. CT has been viewed as the gold standard for evaluation and quantification of VAT.

The basic principle behind CT is the photoelectron effect in which some energy of photon is absorbed by a given object and some of the energy is partially emitted. This emitted energy depends upon the radio-density of the material and can be captured by the detector.

III.A General Process of image construction

The formation of CT images involves 3 steps: data acquisition, image reconstruction and image display. Data acquisition refers to X-ray transmission measured from the patients. During scanning, x-ray tube and detectors rotate around the patients and detectors measure the radiation transmitted through the patient from various locations. X-ray can be attenuated because of the photoelectric effect or, they can be attenuated by the Compton Effect (scattering due to decrease in energy). Since attenuation of a

tissue is a result of absorption and scattering of photons, attenuation measurements can be made from this data. X-ray attenuation can be measured in Hounsfield Unit (HU). Hounsfield units scale is a linear transformation of the original linear attenuation coefficient measurement into one in which the radiodensity of distilled water at standard pressure and temperature is defined as zero HU, while the radiodensity of air at standard pressure and temperature is defined as -1000 HU. Thus, for a matter of interest (in our case, visceral fat) with linear attenuation coefficient μ_{VAT} , the corresponding HU can be given by

$$HU = (\mu_{VAT} - \mu_{Water}) / (\mu_{Water} - \mu_{Air}) \times 100$$

Where μ_{Water} and μ_{Air} are the linear attenuation coefficient of water and air, respectively. This difference in attenuation of various tissues is utilized to characterize and measure tissue of interest.

Reconstruction is an important step in the production of final images where two or three dimensional images are reconstructed by using a large number of projections from different locations. After reconstruction, final images can be viewed, manipulated, and processed on a viewing station. Data acquisition and reconstruction are crucial steps in ensuring high image quality. I will review some key factors that are associated with image quality.

Image Quality

Prior to utilizing any imaging modality in a research project, it is crucial to understand the factors that may influence the quality of data, in our case, image quality.

Images obtained from a CT scanner depends upon various factor, some of which are inherent to a specific machine and others that can be controlled by the technicians such as x-ray tube voltage, tube current, slice thickness, reconstruction parameters.

Parameters that describe the image quality include spatial resolution, temporal resolution, accuracy, artifacts and noise.

Spatial resolution refers to the scanner's ability to resolve closely placed objects. Many factors may affect the spatial resolution such as x-ray focal spot size and shape, detector cell size, scanner geometry, and sampling frequency. Small spot size utilizing lower tube current can offer more spatial resolution. Reconstruction algorithm can also influence image quality by selecting convolution algorithm (kernel). Convolution algorithm has been developed for anatomic-specific application, generally using standard algorithm for soft tissues and bone algorithm for bony structures. Field of view (FOV), pixel size and matrix size are the parameters that greatly affect the spatial resolution. Pixel size is the sampling interval that allows us to visualize small objects. Smaller the pixel size, higher the spatial resolution. The relationship between FOV, pixel size and matrix can be shown by following equation.

$$\text{Pixel Size} = \text{FOV} / \text{Matrix size}$$

For instance, if we have a matrix of 512x 512, and use the FOV of 50 cm, then our pixel size would be 0.98mm, however, if we narrow the FOV to 10 cm, our spatial resolution will improve with pixel size of 0.20mm.

Visibility of an object depends not only on its size but also on its contrast. For our research, we are primarily interested in visualization and measurement of visceral fat which is a low contrast agent given fat's low density and atomic number. CT can detect these low contrast objects from other objects. Low contrast detectability (LCD) of a scanner is defined as the smallest object that can be visualized at a given contrast level and dose. LCD of a system is depends upon the presence of noise. Generally speaking, there are 3 major noise sources; quantum noise, electronic noise and noise due to reconstruction parameters. Electronic noise is usually due to inherent physical limitation of the system. Kernel selection as noted above can affect the noise and subsequently can affect the spatial resolution. Quantum noise is influenced by the scanning techniques such as tube voltage, tube current, slice thickness, scan speed, patient size. It is a complex process as there are tradeoffs with using a certain parameters. For instance, if one increases the tube current, noise can be reduced but dose to the patient is significantly increased. Similarly, if one increase the tube voltage, noise is reduced but the quality of image in detecting low contrast agents is compromised. Increasing the slice thickness can also decrease the noise while decreasing the visibility of small objects. Temporal resolution is essentially CT scanner's

ability to freeze motion of moving objects which is mostly an issue with imaging moving objects such as heart.

For our purpose, visceral fat measurements of MESA participants were performed using helical CT with tube voltage of 120 kV, current of 250mA. We used 4 x 2.5 mm collimation, standard reconstruction kernel, and a display field of view of 500 mm.

Three 5 mm slices were obtained that were centered at the Lumbar spine L4 –L5 level.

Radiation

Due to the fact that CT scanning utilizes ionizing radiation and noted to detrimental health outcomes, it is crucial to understand the source of radiation during CT scanning. Radiation in CT can be expressed in exposure, absorbed dose and effective dose and is expressed in coulombs/Kg, Grays and Sieverts respectively. The total amount of radiation that patient receives is best quantified by the effective dose which is related to the risk of carcinogenesis and genetic effects.

Several factors can directly affect the radiation dose needed in a scan. These factors include exposure technique factors, x-ray beam collimation, pitch, patient centering, and number of detectors. Exposure technique factors are tube current, tube voltage and exposure time. Radiation dose is directly proportional to the current and time which is usually denoted as milliamperage-seconds mAs.

Radiation dose \propto tube current (in milliamps) x time (in secs)

Automatic tube current modulation is often used to minimize tube current in scanner. Similarly, peak tube voltage or potential determines the energy of photons. In this case, radiation dose is proportional to the square of the tube potential.

$$\text{Radiation dose} \propto (\text{peak tube potential})^2 \quad (\text{in kVp})$$

Collimation is used to define the beam width set for the examination. Use of wider collimator can also help in reducing the radiation dose as it radiates more of area that is used for image reconstruction.

Pitch is a term used in helical/spiral CT and is defined as the ratio of the distance the table moves per rotation to the total collimated x-ray beam width.

$$\text{Radiation dose} \propto 1/\text{Pitch}$$

No of detectors is scanner specific and affect the radiation dose in following manner

$$\text{Radiation dose} \propto 1/\text{no. of detectors}$$

Further, it is important that patient is centered in the CT scanner. Improper centering can not only increase radiation dosing, it also increases undesired noise.

Measurement of Visceral Adipose Tissue

Once the image acquisition is complete, visceral adipose tissue volume is determined using image processing and analysis software. On the sagittal image space between L4-L5 vertebrae is located. This space is used as a center point for the slab of slices. First, image area outside the abdomen is chosen to exclude phantom and table.

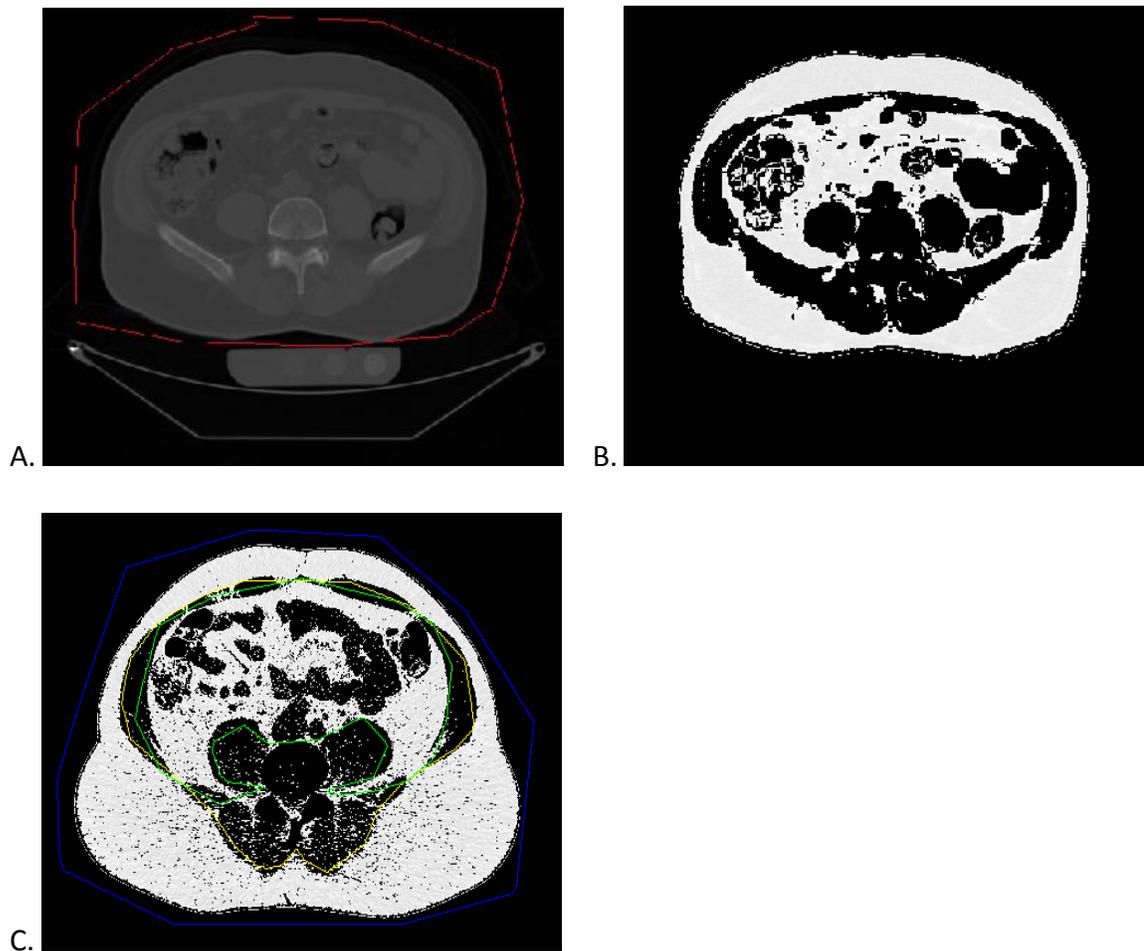


Fig 5: Measurement of Visceral Adipose Tissue using computed tomography. A) Selection of VOI to exclude table and phantom. B) Image only containing abdomen. C) VOIs of abdomen, abdomen plus intermuscular region, and visceral fat.

Volume of interest (VOI) are then drawn around the abdomen, around the visceral plus intermuscular region of the abdomen and finally around the visceral fat. Volume of visceral fat is then calculated by the analysis software based on these VOIs.

While above protocol was used in MESA participants, other investigators have used slightly different approach although fundamental principles behind the analysis remain the same. Figure below shows a method for determining the adipose tissue area on a CT scan. A region of interest of the subcutaneous fat layer is defined by tracing its contour on each scan, and the attenuation range of CT numbers (in Hounsfield units) for fat tissue was calculated (Fig 1, part *a*). A histogram for fat tissue was computed on the basis of mean attenuation plus or minus 2 SD (Fig 1, part *b*). Intraperitoneal tissue is defined by tracing its contour on the scan (Fig 1, part *c*); within that region of interest, tissue with attenuation within the mean plus or minus 2 SD was considered to be the visceral fat area (Fig 1, part *d*). The pixels with attenuation values in the selected attenuation range are depicted as white. From those white regions, the total fat area is calculated by counting the number of pixels in each; the visceral fat area is subtracted, and the remainder was defined as the subcutaneous fat area. A cutoff value of 130 cm² has been proposed as upper limit of normal using ROC curves (110).

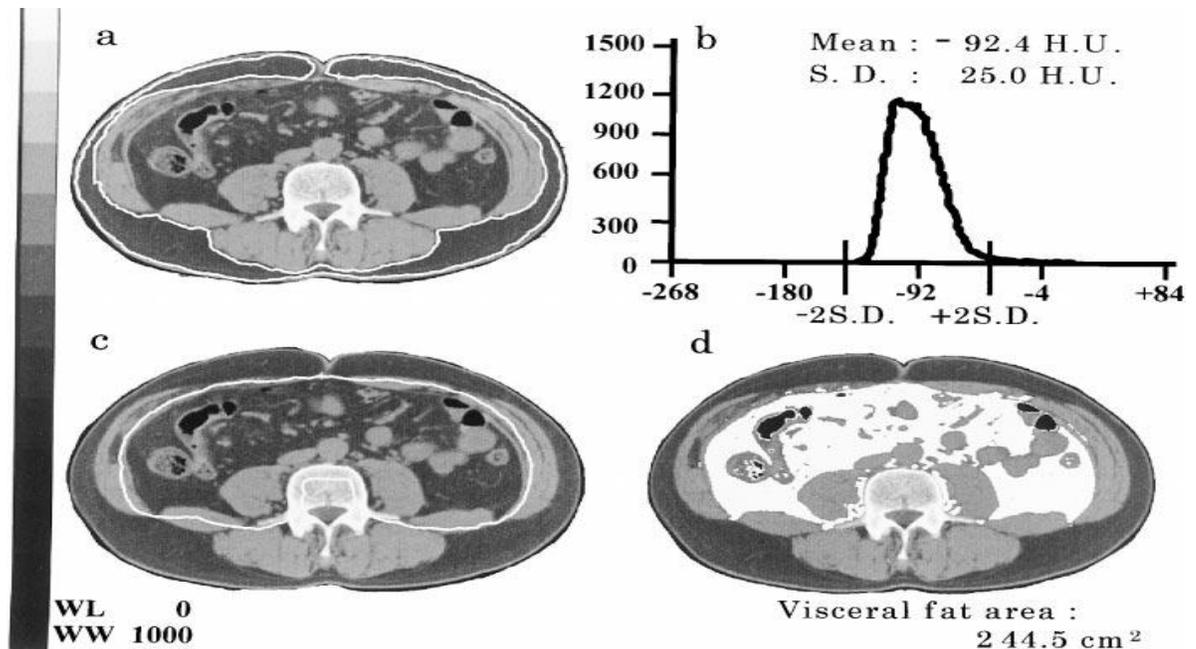


Fig 6. Measurement of Visceral Adipose Tissue. a) A region of interest of the subcutaneous fat layer is defined by tracing its contour on each scan. b) A histogram for fat tissue on the basis of mean attenuation ± 2 SD. c) Tracing of Intraperitoneal tissue. d) Visceral fat within intraperitoneal tissue defined by tissue with attenuation within the mean ± 2 SD. Reprinted with permission. Yoshizumi et al (110)

This quantification of visceral fat by CT scan has been well studied and has been validated. Rossner et al (111) measured adipose tissue surfaces in 11 slices (± 5 cm from the umbilicus) in two cadavers using computed tomography (CT) versus planimetry of band-sawed slices of the corresponding sections. A very close correlation was found with partial correlations of around 0.90. The results were similar whether fat was defined as -250 to -50, -190 to -30, or -140 to -40 Hounsfield units. They concluded that CT measurements agree closely with a direct morphometric method. Others have reached similar conclusion (25,26,112). Intra-observer variation has been noted to be small ranging from 0.2%– 0.8%. Similarly, inter-observer coefficient of variation has also

been noted to be small at 2-3%. Further, inter-equipment variation has shown almost identical estimation by five different CT scanners (110,113,114). Similar to MRI, single slice methods have been investigated by several investigators (115-117). However, which slice to use remains unclear. Kuk et al (118) in their investigation noted that site of measurement matter when calculating visceral adipose tissue. In their study investigating association of metabolic syndrome and visceral fat in men, they found that partial VAT volumes at L1–L2 were higher than volume at the L4–L5 level.

Thus, CT scan is fast, accurate and readily available imaging modality for assessment of visceral adipose tissue. It is considered the gold standard for measurement of visceral adipose tissue. However, ionizing radiation and expense may limit its use in large population based study.

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Curriculum Vitae
Manish Jain, M.D.
mjain@wfubmc.edu

EDUCATION AND TRAINING

1996- 2000 University of Houston

Houston, Texas

Bachelor of Science, Magna Cum Laude, May 2000

2000- 2004 University of Texas Southwestern Medical Center

Dallas, Texas

M.D. – May 2004

2004- 2005 Internship, Internal Medicine

University of Texas Southwestern Medical Center

Dallas, Texas

2005- 2007 Residency, Internal Medicine

University of Texas Southwestern Medical Center

Dallas, Texas

2007-2008 Research Fellow

Institute of Exercise and Environmental Medicine

Presbyterian hospital/University of Texas Southwestern Medical Center

Dallas, Texas

2008- pres. Graduate Student

Clinical Epidemiology and Health Services Research

Wake Forest University Graduate School of Arts and Sciences
Winston-Salem, North Carolina

2008- pres. Cardiology Fellow

Wake Forest Baptist Hospital, Section on Cardiology
Winston-Salem, North Carolina

**AWARDS AND
FUNDING**

1997-2000 Dean's List

University of Houston

2000 Graduation Honors, Magna Cum Laude

University of Houston

2005 Teaching Intern of the year

University of Texas Southwestern Medical Center

2008- pres. Recipient T32 Grant—CVD Epidemiology Training Program

Wake Forest University Health Sciences

PUBLICATIONS

Qi Fu, VanGundy, Tiffany, Galbreath, M, Shibata, Shigeki, Jain, Manish, Hastings, Jeff, Bhella, Paul, Levine, Benjamin. Cardiac Origins of the Postural Orthostatic Tachycardia Syndrome: *Journal of American College of Cardiology*. 2010; 55: 2858-2868.

M Jain, M.D., J. Ding, Ph.D., M. Allison, M.D., M. Criqui, M.D., J Carr, M.D., and D.M.

Herrington, M.D. Association of Visceral Adipose Tissue with Pulse Pressure in the Multi Ethnic Study of Atherosclerosis Pressure (MESA). Abstract accepted for poster presentation at American Heart Association Epidemiology and Prevention (AHA-EPI). 2010.

M. Jain, M.D., D.M. Herrington, M.D., W.G. Hundley, M.D., David A. Bluemke, M.D., and A. Bertoni, M.D. Association of Physical Activity with Pulse Pressure in the Multi-Ethnic Study of Atherosclerosis (MESA). Abstract accepted for poster presentation at American Heart Association Epidemiology and Prevention (AHA-EPI). 2010.

Jain M, Shah M, Matulevicius S, Peshock R. Correlation between diastolic dysfunction and aortic compliance in Dallas Heart Study population using peak filling rate as a measure of diastolic dysfunction by cardiac magnetic resonance imaging. Abstract accepted for oral presentation at American Heart Association 2007.

Jain M, Matulevicius S, Leonard D, Shah M, Peshock R. Prediction of Left Ventricular Peak Filling Rate by Three Short Axis Cardiac Magnetic Resonance Imaging Slices – Accepted for poster presentation at Society of Cardiovascular Magnetic Resonance(SCMR), Jan 2008.

**PROFESSIONAL
ORGANIZATIONS**

American Heart Association (AHA)

American Heart Association- Epidemiology Counsel

American College of Cardiology Fellows in Training (ACC-FIT)

American medical Association (AMA)