The Association Between Subcutaneous Fat Density and the Propensity to Store Fat Viscerally

Title Page

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Student Name: Aaron J. Yeoh
Scholarly Report Title: The Association Between Subcutaneous Fat Density and the Propensity to Store Fat Viscerally

Mentor Name(s) and Affiliations: Caroline S. Fox[1,2]
[1]National Heart, Lung and Blood Institute, Framingham Heart Study, Framingham, Massachusetts; [2]Division of Endocrinology and Metabolism, Brigham and Women's Hospital and Harvard Medical School, Boston Massachusetts

Collaborators, with Affiliations: Alison Pedley,[1] Klara J. Rosenquist,[1] Udo Hoffmann,[3]
[1]National Heart, Lung and Blood Institute, Framingham Heart Study, Framingham, Massachusetts; [3]Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts

Abstract

TITLE: The Association Between Subcutaneous Fat Density and the Propensity to Store Fat Viscerally.

Yeoh AJ, Pedley A, Rosenquist KJ, Hoffmann U, Fox CS.

BACKGROUND:

Alterations in the cellular characteristics of subcutaneous adipose tissue (SAT) may reduce its ability to expand in times of caloric excess, increasing the propensity to store excess calories viscerally (visceral adipose tissue [VAT]). We hypothesized (1) that increased SAT density, an indirect marker of fat quality, would be associated with an increased VAT/SAT ratio and increased cardiovascular disease (CVD) risk and (2) that these associations would be independent of the absolute volume of SAT.

METHODS:

We investigated the association of SAT density with the VAT/SAT ratio and CVD risk in 3212 participants (48% women, mean age, 50.7 years) from the Framingham Heart Study. Adipose tissue depot density and volume were quantified by computed tomography; traditional CVD risk factors were quantified.

RESULTS:

Higher SAT density was correlated with a higher VAT/SAT ratio in men ($r = 0.17$; $P < .0001$) but not in women ($r = 0.04$; $P \geq .05$). More adverse levels of CVD risk factors were observed in the high SAT density/high VAT/SAT ratio group than in the referent group (low density/low ratio). For example, women had an increased risk of diabetes (odds ratio [OR], 6.7; 95% confidence interval [CI], 2.6-17.6; $P = .0001$) and hypertension (OR, 1.6; 95% CI, 1.1-2.4; $P = .009$). Additional adjustment for SAT volume generally strengthened these associations (diabetes OR, 10.8; 95% CI, 4.1-29.0; hypertension OR, 2.5; 95% CI, 1.7-3.7; all $P < .0001$). These trends were similar but generally weaker in men.

CONCLUSION:

High fat density, an indirect marker of fat quality, is associated with the propensity to store fat viscerally vs subcutaneously and is jointly characterized by an increased burden of CVD risk factors.

Contribution to Work

In the summer of 2013, I spent 10 weeks working with Dr. Caroline Fox at the Framingham Heart Study, a project through the National, Heart, Lung, and Bloody Study of the National Institute of Health. Before this summer work started, I did a literature review pertinent to my work in order to better organize the goals of the project.

In the first few weeks of the summer, Dr. Fox and I met several times to clarify our hypotheses. I reviewed the large data set collected from 2002-2005 of which included CT scan data, CVD risk factors, and covariate data from participants of the Framingham Heart Study that would serve as the basis our inquiry.

From there, we began weekly meetings with our statistician, Dr. Alison Pedley to build the regression models used to test the association between SAT density and the VAT/SAT ratio, our primary outcome for our study. We built additional models to explore the relationship between the VAT/SAT ratio and CVD risk outcomes with adjustments for age, sex, alcohol use, smoking status, physical activity, and hormone replacement therapy. We spent about 6 weeks working with Dr. Alison Pedley, examining and revising our models each week until our outputs were appropriate for our project aims. During this period, I wrote the Methods and Results section of the manuscript and was responsible for making all tables included in the paper.

Throughout the middle 6 weeks of my summer work, I also spent time collecting CT image data by drawing ellipse lines in the area of interest and determined tissue density of this area radiographically. This was a similar technique used to acquire the CT image data that was used for my project and provided me with an understanding of how that data I used for my project was obtained and refined. This separate CT data was utilized for another project with Dr. Fox and one of her Fellows.

In the final weeks of my summer work, I extended my review of the literature in order to write a thorough Introduction and Discussion sections of my manuscript. I continued to meet regularly with Dr. Fox as I drafted the manuscript and made changes based her feedback. We submitted the paper in the fall and I helped with issues raised in the single revision required by the journal. The paper was published the following year

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Appendix 1: Final Manuscript Before Acceptance by JCEM Journal

Full Title: The Association between Subcutaneous Fat Density and the Propensity to Store Fat Viscerally

Authors: Aaron J. Yeoh,[1] Alison Pedley,[1] Klara J. Rosenquist,[1] Udo Hoffmann,[3] & Caroline S. Fox[1,2]

[1]National Heart, Lung and Blood Institute, Framingham Heart Study, Framingham, Massachusetts; [2]Division of Endocrinology and Metabolism, Brigham and Women's Hospital and Harvard Medical School, Boston Massachusetts [3]Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts

Abbreviated Title: Association of SAT density and the VAT/SAT ratio

Keywords: Obesity; Subcutaneous fat; Visceral fat; Fibrosis; Risk factors

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Corresponding Author's Contact Information: Dr. Caroline S. Fox, NHLBI'S Framingham Heart Study 73 Mt. Wayte Avenue, Suite 2, Framingham, Massachusetts 01702. Email: foxa@nhlbi.nih.gov

Reprint Requests: Dr. Caroline S. Fox, NHLBI'S Framingham Heart Study 73 Mt. Wayte Avenue, Suite 2, Framingham, Massachusetts 01702

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Abstract (Word count 250)

Context: Alterations in the cellular characteristics of subcutaneous adipose tissue (SAT) may reduce its ability to expand in times of caloric excess, increasing the propensity to store excess calories viscerally (VAT).

Objective: We hypothesized that increased SAT density, an indirect marker of fat quality, would be associated with 1) an increased VAT/SAT ratio and increased CVD risk and 2) that these associations would be independent of the absolute volume of SAT.
Design and Setting: We investigated the association of SAT density with VAT/SAT ratio and CVD risk in the Framingham Heart Study.
Participants: 3212 participants (48% women, mean age 50.7 years)
Intervention: None
Main Outcome Measures: Adipose tissue depot density and volume were quantified by computed tomography; traditional cardiovascular disease (CVD) risk factors were quantified.
Results: Higher SAT density was correlated with higher VAT/SAT ratio in men ($r = 0.17$; $p<0.0001$) but not in women ($r=0.04$; $p>0.05$). More adverse levels of CVD risk factors were observed in the high SAT density/high VAT/SAT ratio group compared with the referent group (low density/low ratio). For example, women had an increased risk of diabetes (odds ratio [OR]: 6.7; 95% CI: 2.6-17.6; $p=0.0001$) and hypertension (OR:1.6; 95% CI: 1.1-2.4; $p=0.009$). Additional adjustment for SAT volume generally strengthened these associations (diabetes OR:10.8; 95% CI:4.1-29.0; hypertension OR:2.5; 95% CI:1.7-3.7; all $p<0.0001$). These trends were similar but generally weaker in men.
Conclusion: High fat density is associated with the propensity to store fat viscerally vs. subcutaneously, and jointly characterized by an increased burden of CVD risk factors.

Introduction

Body mass index (BMI) and waist circumference have been used to assess obesity, but these measurements contain little information about the anatomic location of stored excess fat. Regional fat depots may confer differential cardiovascular disease (CVD) risk, with visceral adipose tissue (VAT) volume more pathogenic than subcutaneous adipose tissue (SAT) volume (1). A large body of work has shown that VAT is associated with increased insulin resistance, type 2 diabetes, metabolic syndrome and the atherogenic lipoprotein profile (2-7).

In addition to fat volume, previous studies have focused on aspects of fat quality, including cellular characteristics biopsied invasively (8-14). Fat quality may be an important metric to characterize adipose tissue with respect to cardiovascular and metabolic risk, but can be difficult to capture in free-living populations. We have previously shown that fat quality can be indirectly estimated using the density of adipose tissue as measured radiographically on computed topography (CT) with Hounsfield Units (HU) (15). Small experimental studies in the

setting of brown adipose tissue suggest that fat attenuation on computed tomography may reflect extracellular matrix (ECM) remodeling with high HU associated with increased metabolic activity and low HU associated with more lipid dense adipocytes (16;17). SAT fibrosis may be reflected by a relatively high density of fat tissue, and thus estimated by the higher HU (15). We hypothesized that when SAT becomes fibrotic, the adipocytes cannot expand in times of calorie excess, forcing excess calories to be stored in VAT, which may be metabolically maladaptive. This shift in fat stores of the body from subcutaneous to visceral depots may be reflected by a high VAT/SAT ratio. The VAT/SAT ratio has been associated with CVD and metabolic risk, beyond BMI or VAT alone (2;3;5;18;19). Thus, the goal of our study was to 1) examine whether increases in SAT density were associated with a propensity to store fat viscerally vs. subcutaneously and 2), to test whether high fat density and a high VAT/SAT ratio are jointly associated with an increased burden of CVD risk factors.

Methods

Study Sample

All participants for this study were drawn from the Framingham Heart Study. The Original cohort and their spouses were enrolled in 1948, and their children were enrolled as the Offspring cohort in 1971 and finally the Third Generation cohort was enrolled in 2002 (20-22). Data for this current project was collected from the Offspring cohort and the Third Generation cohort enrolled in a multidetector computed tomography (MDCT) sub-study (23). The MDCT subcohort contains primarily individuals residing in the United States New England area. Participants were eligible if they were female (>40 years of age), male (>35 years of age), had a body weight of <160 kg and were not pregnant. Between 2002 and 2005, a total of 3,539 participants (1,422 from Offspring cohort and 2,117 from the Third Generation cohort) underwent CT scans of the abdomen. Of these 3539 participants, 3394 had acceptable quality CT data. Participants with missing outcomes (VAT and SAT volumes and HU) or missing covariates were excluded, resulting in a final sample size of 3212 (48% women). Informed consent was required for each participant and this study met the terms of the Declaration of Helsinki and was authorized by the Institutional Review Board of the Boston University Medical Center.

CT Measurements

Participants underwent supine eight-slice MDCT scans of the abdomen for SAT, VAT and other adipose tissue depots resulting in 25 contiguous 5-mm slices. SAT and VAT volumes were manually outlined by drawing the line of the abdominal muscular wall separating these two depots. Fat depots were designated for the area with an HU measurement between -195 and -45 HU. This technique demonstrates highly reproducible inter-reader and intra-reader correlations (0.997 for SAT and 0.992 for VAT) (24). Further work evaluated the variability within each depot using 1 cm^2 traces from 12 anatomically distinct regions in three slices per individual from 25 randomly selected participants. The average standard deviation for SAT and VAT was 7.6 HU and 5.5 HU respectively, suggesting only modest variability of HU measurements within each depot (15).

CVD Risk Factor Assessment

CVD risk factor data was obtained from the seventh examination of the Offspring cohort and the Third Generation cohort. Weight was assessed on a Detecto (Webb City, Missouri) scale during the examination and waist circumference was measured with a standard tape measure at the level of the umbilicus to the closest ¼ inch. Body mass index (BMI) was defined as weight (kg) divided by square of height (m^2). Participant blood pressure was measured at rest twice with a mercury column sphygmomanometer and hypertension was defined as a systolic pressure ≥ 140 mm Hg or a diastolic pressure ≥ 90 mm Hg or the routine use of hypertensive medication. Serum metabolic risk factors, including plasma glucose, total cholesterol, HDL cholesterol and triglycerides, were assessed based on participant's fasting blood samples. Diabetes was defined as fasting blood glucose of ≥126 mg/dl or treatment with insulin or hypoglycemic agent. Metabolic syndrome was classified based on the adapted Adult Treatment Panel III criteria (25).

Covariates

Questions administered by examining physicians were used to assess smoking and alcohol intake status. Current smokers were defined as individuals that had smoked at least one cigarette per day for the last year. Alcohol intake was dichotomized based on the criteria for men (>14 drinks/week) and women (>7 drinks/week). Physical activity score was determined

from a questionnaire that assessed daily activity levels and average number of hours slept. Women were classified whether they received hormone replacement therapy (HRT).

Statistical Analysis

Fat density and VAT/SAT ratio were selected as the primary exposures in this study. SAT HU was dichotomized at the median value (cutoff value of -103.5 in women and -100.3 in men). The VAT/SAT ratio outcome was dichotomized at the median value (cutoff value of 0.3946 in women and 0.838 in men). SAT HU, an indirect marker of tissue density, was renamed for our analysis as either high or low density, and the VAT/SAT ratio was simplified to high or low ratio.

Four groups were created from our dichotomized primary exposure and outcome: 1) high density/high ratio (ie high SAT density with a high propensity to store fat viscerally), 2) high density/low ratio (high SAT density with a low propensity to store fat viscerally), 3) low density/high ratio (low SAT density with a high propensity to store fat viscerally) and 4) low density/low ratio (low SAT density with a low propensity to store fat viscerally). We hypothesized *a priori* that the high density/high ratio group would have the most adverse CVD risk factor profile. For comparative clinical analyses between groups, we designated the low density/low ratio group as our metabolically healthy, referent group. Because of the known sexual dimorphism for in fat distribution, all models were stratified by sex.

The VAT/SAT ratio was log transformed as necessary to produce a more normal distribution. We first computed age-adjusted correlations between the log of the VAT/SAT ratio and CVD risk factors. Next, multivariable logistic regression was used to test the association between SAT density (high versus low) and VAT/SAT ratio (high versus low). Finally, multivariable-adjusted logistic and linear regression models were used to test the association of each group compared with the referent group and CVD risk outcomes. Model 1 adjusted for age, sex, alcohol use, smoking status, physical activity, and hormone replacement therapy (women). Model 2 additionally adjusted for SAT volume (continuous).

Results

Study Sample Characteristics

Clinical characteristics of the 3212 study participants are presented in Table 1, stratified by the four groups. In women, mean BMI ranged from 24.7 kg/m^2 in the high density/low ratio group up to 28.4 kg/m^2 in the low density/high ratio group. A similar pattern was seen for mean absolute SAT volume with a range of 2472 cm^3 to 3908 cm^3 in the high density/low ratio and low density/low ratio groups, respectively. In men, BMI and SAT followed the same pattern. In general, participants with high density/high ratio or low density/high ratio had the most adverse levels of CVD risk factors.

As the VAT/SAT ratio increased, so did CVD risk factors in both women and men with an r value ranging from 0.01 to 0.49 in women and 0.07 to 0.38 in men (Table 2).

Association between SAT density and the VAT/SAT ratio

After adjusting for age, there was an association in men between subcutaneous HU and VAT/SAT ratio ($r = 0.17$, $p < 0.0001$). In women, however, this correlation was not significant ($r = 0.04$, $p \geq 0.05$).

We next utilized multivariable logistic regression to predict high VAT/SAT ratio from dichotomous SAT density. In men, high SAT density was associated with high VAT/SAT ratio (OR: 1.83; 95% CI: 1.49, 2.24; $p < 0.0001$). Results were similar after adjustment for BMI (OR: 1.70; 95% CI: 1.37, 2.09; $p < 0.0001$). In contrast, in women, we found that high SAT density was not associated with high VAT/SAT ratio (OR: 0.89; 95% CI: 0.72, 1.11; $p = 0.31$), even after adjustment for BMI (OR: 0.96; 95% CI: 0.77, 1.21; $p = 0.74$).

Clinical Risk Factors by SAT Density and VAT/SAT Ratio Grouping

As hypothesized, for both women and men, the high density/high ratio group had the most adverse CVD risk factors compared with the referent group. For example, in women, the high density/high ratio group had an increased odds of hypertension (OR: 1.6; 95% CI: 1.1-2.4; $p = 0.009$, Table 3), diabetes (OR: 6.7; 95% CI: 2.6-17.6; $p = 0.0001$, Table 3) and fasting glucose (4.8 mg; 95% CI: 2.2-7.4; $p = 0.0004$, Table 4). When we further adjusted our models for absolute SAT volume, results were generally stronger. For example, the high density/high ratio group had an increased odds ratio hypertension (OR: 2.5; 95% CI: 1.7-3.7; $p < 0.0001$, Table 3), diabetes (OR: 10.8; 95% CI: 4.1-29.0; $p < 0.0001$, Table 3) and fasting glucose (8.9

mg; 95% CI: 6.2-11.5; p < 0.0001, Table 4). In men, similar patterns were observed, although results were less striking. However, with additional adjustment for SAT volume, results were generally stronger (hypertension OR: 1.5; 95% CI: 1.0-2.1; p = 0.03; diabetes OR: 4.8; 95% CI: 2.6-9.2; p < 0.0001; fasting glucose 10.0 mg; 95% CI: 6.5-13.5; p < 0.0001).

The low density/high ratio group had a similar but less adverse profile than the high density/high ratio group. For example, in women, compared with the referent group, the low density/high ratio group had increased hypertension (OR: 1.8; 95% CI: 1.3-2.5; p = 0.001) and high triglycerides (OR: 1.7; 95% CI: 1.2-2.3; p = 0.003). After SAT adjustment, findings were generally stronger.

The high density/low ratio group demonstrated more variable results with both multivariable and SAT-adjusted multivariable models. This group generally had a less adverse CVD risk profile compared with the referent group in both sexes. When SAT-adjusted, this group still had the least adverse CVD risk profile compared to the referent group but the difference was attenuated. There was a notable exception of greatly increased risk of diabetes with this group compared to the referent group after SAT adjustment (Women OR: 4.2; 95% CI: 1.4-12.5; p = 0.01; Men OR: 2.6; 95% CI: 1.4-4.9; p = 0.004).

Finally, when multivariable models were additionally adjusted for absolute VAT volume in addition to absolute SAT volume, most findings were attenuated. Notably, there was an exception with diabetes in women in the high density/high ratio group compared with the referent group (OR: 3.5; 95% CI: 1.1-10.4; p = 0.03) and men (OR: 2.7; 95% CI: 1.3-5.5; p 0.008; data not shown).

Discussion

Principal Findings

Our principal findings are three-fold. First, high SAT density was associated with high VAT/SAT ratio in men, but not women. Second, the high density/high ratio group was associated with the most adverse CVD risk factor levels in women and men. Finally, these results were generally strengthened after adjustment for absolute SAT volume. Taken together, these findings suggest that increased SAT density along with a high VAT/SAT ratio is associated with a heavy burden of CVD risk factors.

In the *Context of the Current Literature*

In a state of positive caloric balance, excess free fatty acids are stored in adipose tissue, preferentially in the subcutaneous adipose depot (26). Remodeling of adipose tissue occurs during times of caloric excess via hypertrophy of adipocytes and hyperplasia of pre-adipocytes into mature adipocytes, as well as changes in the number of stromal and immune cells involved with adipose tissue function (27-29). Adipocyte tissue remodeling is a constant process that may become pathologic in an obese state, with features such as hypoxia, reduced angiogenesis, and invasion of immune cells with a subsequent pro-inflammatory response and overproduction of ECM (9-13;30). When adipose tissue reaches maximum expansion potential, excess free fatty acids overflow to ectopic sites, (31). Alternatively, maximum expansion potential may be reduced, leading to lower effective fat storage and an increased propensity for fat spillover to ectopic sites.

The reduction in maximum SAT expansion potential may be due to at least four interconnected mechanisms seen in pathologic obesity: 1) reduced angiogenesis, 2) inflammation, 3) fibrosis, and 4) fibrosis-driven physical remodeling. Excess caloric intake drives the rapid expansion of adipose tissue. Adipose tissue can become hypoxic if the vasculature cannot keep pace with tissue growth, leading to increased levels of hypoxia-inducible factor 1α (HIF1α) (32-34). HIF1α mediates induction of macrophage proinflammatory cytokines as well as increased expression of fibrotic response genes (32;34). Mice lacking a form of collagen highly enriched in adipose tissue IV exhibit uninhibited adipocyte expansion, suggesting the importance of fibrosis in reducing SAT expansion potential (35;36). Mechanical compression of human adipose tissue in 3D culture, a model for the physical role of fibrosis on adjacent adipocytes, further induces proinflammatory and fibrotic gene expression of adipocytes (37). Thus, increasing hypoxia, inflammation, fibrosis and fibrosis-driven remodeling may drive a reduction in SAT storage capacity.

In the present study, we used high SAT density as a marker of increased adipose tissue fibrosis. We hypothesized that as SAT becomes increasingly fibrotic, adipocytes cannot expand in times of caloric excess, causing fat spillover into the ectopic VAT depot. It is now well established that an excess of VAT in obese and non-obese individuals is associated with increased cardiovascular and metabolic abnormalities (38-41). A small number of studies have reported an association between VAT/SAT ratio and CVD risk factors, with increasing VAT/SAT ratio associated with increasing CVD risk (2;3;5;18;19). We now expand the literature by

demonstrating an association between SAT density and the VAT/SAT ratio, and that jointly, those with high density and a high ratio carry a high burden of CVD risk factors. In particular, our results for diabetes are notable, even after we statistically adjusted for variation across the four categories in SAT and VAT. This may be consistent with a mechanism of increased adipose tissue fibrosis leading to diabetes. In mouse models, overexpression of HIF1α initiated adipose tissue fibrosis and insulin resistance (33). Moreover, mice lacking collagen VI, the major collagen form seen in adipose tissue fibrosis, exhibit increased insulin sensitivity, suggesting the importance of fibrosis in determining insulin resistance (35;36). Furthermore, the VAT/SAT ratio was positively associated with fasting glucose in a group of adults and with hepatic insulin resistance in a group of men with type 2 diabetes (whereas VAT or SAT alone were not) (2;3). Thus, this work improves the framework with which to interpret abdominal CT scan data as it relates to CVD risk factors.

Implications for Future Work

Our findings suggest that cellular characteristics of adipose tissue, indirectly measured using fat density, may be an important mechanism contributing to relative fat deposition and concomitant metabolic risk. While our data suggests an association between SAT density and VAT/SAT ratio, the mechanism linking these two measurements remains uncertain. Furthermore, it is not clear why individuals with high density and high VAT/SAT ratio have the most adverse CVD risk factor profile. These questions warrant further investigation, particularly in studies that correlate adipose tissue imaging with invasive biopsy of adipose tissue and subsequent histologic and biochemical work to elucidate the underlying mechanisms of adipose tissue remodeling.

Strengths and Limitations

Strengths of our study include a highly specific and reproducible CT-derived assessment of fat volume and quantity. In addition, we made use of the large, well-defined Framingham Heart Study cohort that undergoes regular examinations and provides high quality biochemical and clinical data. Some limitations of this study include the cross-sectional design, which limits inferences of temporality. Moreover, the observational nature of the data limits causality to be concluded from our results. Our cohort is primarily non-Hispanic White, which may limit the

generalizability of our findings. Finally, the underlying biological mechanism of fat density is not well defined; therefore further laboratory studies are needed.

Conclusion

Increased subcutaneous adipose tissue fat density is associated with an increased propensity to store excess fat viscerally rather than subcutaneously, resulting in an increased VAT/SAT ratio. High SAT density and high VAT/SAT ratio are jointly characterized by an increased burden of CVD risk factors.

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Table 1: Clinical characteristics of study participants, subdivided into four groups: high density/high ratio, high density/low ratio, low density/high ratio, low density/low ratio.* For continuous data, SD is provided in parentheses. For dichotomous data, the numerator is provided in parentheses.

	High Density/ High Ratio	**High Density/ Low Ratio**	**Low Density/ High Ratio**	**Low Density/ Low Ratio**
Women (n)	363	397	400	366
Age (years)	55.8 (11.1)	47.3 (7.5)	55.2 (9.5)	49.3 (7.6)
Smoking (%)	15.4 (56)	9.3 (37)	13.8 (55)	9.8 (36)
Moderate – Heavy Alcohol Use (%)	16.5 (60)	14.9 (37)	17.5 (70)	10.4 (38)
Physical Activity Score (%)	37.8 (6.1)	37.0 (6.1)	36.2 (5.5)	36.0 (5.4)
Total Cholesterol (mg/dl)	198.7 (36.9)	185.0 (32.7)	208.2 (39.0)	199.0 (32.2)
HDL Cholesterol (mg/dl)	58.6 (16.6)	67.4 (17.6)	58.1 (16.4)	60.5 (14.9)
Low HDL (%)	31.4 (114)	15.4 (61)	32.3 (129)	24.0 (88)
Triglycerides (mg/dl)	130.7 (84.2)	80.0 (42.2)	135.8 (71.9)	107.5 (55.1)
High Triglycerides (%)	37.7 (137)	8.8 (35)	39.0 (156)	21.9 (80)
Fasting Glucose (mg/dl)	100.8 (24.8)	90.5 (15.7)	98.5 (16.2)	93.7 (12.0)
High Glucose (%)	22.3 (81)	6.0 (24)	28.5 (114)	16.7 (61)
Diabetes (%)	13.8 (50)	2.8 (11)	4.3 (17)	1.4 (5)
Hypertension (%)	37.3 (135)	10.9 (43)	38.5 (154)	19.2% (70)
Cardiovascular Disease (%)	7.7 (28)	0.8 (3)	5.5 (22)	1.1 (4)
Metabolic Syndrome (%)	38.0 (138)	7.6 (30)	42.4 (169)	20.9 (76)
Hypertensive Treatment (%)	28.1 (102)	7.1 (28)	27.5 (110)	11.7 (43)
Lipid Treatment (%)	16.5 (60)	3.5 (14)	13.0 (52)	8.5 (31)
Systolic Blood Pressure (mmHg)	123.5 (19.1)	112.6 (13.9)	125.6 (18.7)	119.1 (15.3)
Diastolic Blood Pressure (mmHg)	73.3 (10.1)	70.9 (8.7)	75.9 (8.9)	74.3 (8.1)
VAT/SAT Ratio	0.6 (0.3)	0.3 (0.1)	0.6 (0.1)	0.3 (0.1)

BMI (kg/m^2)	27.1 (6.9)	24.7 (5.6)	28.4 (4.7)	28.2 (5.1)
Waist Circumference (cm)	93.5 (18.1)	84.9 (14.3)	98.3 (12.2)	95.7 (13.4)
Subcutaneous Adipose Tissue (cm^3)	2706 (1604)	2472 (1406)	3515 (1153)	3908 (1438)
Visceral Adipose Tissue (cm^3)	1612 (1020)	706 (461)	1922 (678)	1192 (485)
Subcutaneous HU (cm^3)	-99.0 (5.3)	-98.7 (4.9)	-105.5 (1.5)	-105.8 (1.7)
Visceral HU (cm^3)	-92.3 (4.4)	-89.0 (3.2)	-96.1 (3.3)	-92.5 (3.2)
Men (n)	497	350	346	493
Age (years)	53.1 (11.8)	47.7 (9.7)	51.5 (10.3)	45.9 (8.5)
Smoking (%)	13.1 (65)	16.9 (59)	13.0 (45)	11.4 (56)
Moderate – Heavy Alcohol Use (%)	19.9 (99)	15.1 (53)	18.5 (64)	11.0 (54)
Physical Activity Score (%)	38.9 (8.0)	39.3 (9.0)	37.9 (7.8)	37.4 (8.1)
Total Cholesterol (mg/dl)	193.3 (34.5)	189.9 (32.7)	200.2 (33.0)	196.0 (35.0)
HDL Cholesterol (mg/dl)	44.7 (12.9)	50.4 (13.2)	44.2 (11.0)	45.1 (11.4)
Low HDL (%)	40.1 (199)	17.7 (62)	37.0 (128)	32.7 (161)
Triglycerides (mg/dl)	155.0 (124.9)	107.2 (74.3)	161.7 (113.4)	140.6 (99.6)
High Triglycerides (%)	49.7 (247)	24.9 (87)	55.8 (193)	42.5 (209)
Fasting Glucose (mg/dl)	105.6 (33.2)	100.9 (26.7)	101.0 (11.4)	100.1 (13.7)
High Glucose (%)	36.2 (180)	25.4 (89)	47.0 (162)	39.6 (195)
Diabetes (%)	12.1 (60)	7.1 (25)	3.8 (13)	5.1 (25)
Hypertension (%)	37.8 (188)	18.0 (63)	35.3 (122)	31.2 (154)
Cardiovascular Disease (%)	11.3 (56)	5.4 (19)	9.8 (34)	4.5 (22)
Metabolic Syndrome (%)	38.4 (191)	18.1 (63)	49.4 (171)	42.4 (208)
Hypertensive Treatment (%)	26.2 (130)	11.1 (39)	20.2 (70)	16.4 (81)
Lipid Treatment (%)	20.3 (101)	11.4 (40)	22.0 (76)	16.0 (79)
Systolic Blood Pressure (mmHg)	125.0 (15.9)	119.5 (13.0)	125.7 (14.4)	122.7 (13.8)
Diastolic Blood Pressure (mmHg)	77.3 (9.7)	75.7 (8.6)	79.3 (8.2)	79.7 (9.1)

VAT/SAT Ratio	1.2 (0.4)	0.6 (0.1)	1.1 (0.3)	0.6 (0.1)
BMI (kg/m^2)	27.5 (4.4)	27.0 (4.9)	29.0 (3.6)	30.0 (4.5)
Waist Circumference (cm)	98.0 (11.2)	96.1 (12.7)	103.0 (8.9)	105.4 (11.5)
Subcutaneous Adipose Tissue (cm^3)	1974 (817)	2250 (1211)	2713 (767)	3527 (1252)
Visceral Adipose Tissue (cm^3)	2405 (1074)	1400 (775)	2976 (852)	2108 (733)
Subcutaneous HU (cm^3)	-96.6 (4.0)	-96.3 (4.4)	-102.3 (1.5)	-103.0 (1.7)
Visceral HU (cm^3)	-94.8 (4.1)	-90.7 (3.8)	-98.8 (2.6)	-96.4 (3.3)

*In women, high SAT HU was defined at a cutoff value above -103.6 and high VAT/SAT ratio was defined at a cutoff value of 0.395. In men, the high SAT HU cutoff value was -100.3 and the high VAT/SAT ratio cutoff value was 0.839.

Table 2: Age Adjusted Correlations between log transformed VAT/SAT ratio and selected CVD risk factors for women and men.

	Women (n = 1526)	**Men (n = 1686)**
Age	0.42****	0.37****
Body Mass Index	0.06*	-0.14****
Waist Circumference	0.10****	-0.16****
Systolic Blood Pressure	0.12****	0.08***
Diastolic Blood Pressure	0.11****	0.08***
HDL Cholesterol	-0.24****	-0.16****
Triglycerides (Log)	0.32****	0.22****
Physical Activity Score	0.01	0.07**
Subcutaneous HU	0.04	0.17****
Visceral HU	-0.49****	-0.38****
Subcutaneous Adipose Tissue (SAT)	-0.11****	-0.44****
Visceral Adipose Tissue (VAT)	0.53****	0.42****

*p < 0.05, **p < 0.01, ***p< 0.001, ****p< 0.0001

Table 3: Logistic Regression for Selected CVD Risk Factors as Compared to the Referent Group (Low Density/Low Ratio).*

Dependent Variable		**High Density/ High Ratio**	**p-value**	**High Density/ Low Ratio**	**p-value**	**Low Density/ High Ratio**	**p-value**
Women							
Low HDL	MV	1.6 (1.1, 2.2)	0.01	0.6 (0.4, 0.9)	0.006	1. 7 (1.2, 2.3)	0.003
	MV + SAT	2.4 (1.7, 3.6)	<0.0001	0.9 (0.6, 1.4)	0.70	2.0 (1.4, 2.8)	<0.0001
High Triglycerides	MV	1.5 (1.1, 2.1)	0.02	0.4 (0.2, 0.6)	<0.0001	1.7 (1.2, 2.3)	0.003
	MV + SAT	2.2 (1.5, 3.5)	<0.0001	0.5 (0.3, 0.9)	0.009	2.0 (1.4, 2.8)	0.0001
High Glucose	MV	1.2 (0.8, 1.8)	0.40	0.3 (0.2, 0.6)	<0.0001	1.6 (1.1, 2.4)	0.008
	MV + SAT	1.9 (1.3, 2.9)	0.003	0.6 (0.3, 1.0)	0.04	2.1 (1.4, 3.0)	0.0002
Diabetes	MV	6.7 (2.6, 17.6)	0.0001	2.4 (0.8, 6.9)	0.12	2.0 (0.7, 5.6)	0.18
	MV + SAT	10.8 (4.1, 29.0)	<0.0001	4.2 (1.4, 12.5)	0.01	2.6 (0.9, 7.2)	0.08
Hypertension	MV	1.6 (1.1, 2.4)	0.009	0.6 (0.4. 0.9)	0.01	1.8 (1.3, 2.5)	0.001
	MV + SAT	2.5 (1.7, 3.7)	<0.001	0.9 (0.6, 1.4)	0.63	2.1 (1.5, 3.1)	<0.0001
CVD	MV	3.9 (1.3, 11.6)	0.02	0.8 (0.2, 3.8)	0.82	2.9 (1.0, 8.8)	0.06
	MV + SAT	3.4 (1.1, 10.5)	0.03	0.7 (0.2, 3.4)	0.68	2.8 (0.9, 8.4)	0.07
Metabolic Syndrome	MV	1.7 (1.2, 2.4)	0.005	0.3 (0.2, 0.5)	<0.0001	2.1 (1.5, 3.0)	<0.0001
	MV + SAT	4.8 (3.1, 7.4)	<0.0001	0.8 (0.4, 1.3)	0.30	3.8 (2.6, 5.6)	<0.0001
Hypertensive Treatment	MV	1.9 (1.3, 2.9)	0.002	0.7 (0.4, 1.1)	0.11	1.9 (1.3, 2.9)	0.002
	MV + SAT	2.9 (1.8, 4.5)	<0.0001	1.0 (0.6, 1.7)	0.96	2.3 (1.5, 3.5)	<0.0001
Lipid Treatment	MV	1.3 (0.8, 2.1)	0.32	0.4 (0.2, 0.9)	0.02	1.0 (0.6, 1.7)	0.90
	MV + SAT	1.5 (0.9, 2.6)	0.11	0.6 (0.3, 1.1)	0.09	1.1 (0.7, 1.8)	0.67
Men							
Low HDL	MV	1.5 (1.1, 2.0)	0.005	0.4 (0.3, 0.6)	<0.0001	1.3 (0.9, 1.7)	0.11
	MV + SAT	2.5 (1.8, 3.5)	<0.0001	0.6 (0.4, 0.9)	0.02	1.7 (1.2, 2.4)	0.001

High Triglycerides	MV	1.2 (0.9, 1.5)	0.26	0.4 (0.3, 0.6)	<0.0001	1.5 (1.2, 2.0)	0.003
	MV + SAT	1.9 (1.4, 2.7)	<0.0001	0.6 (0.4, 0.9)	0.005	2.0 (1.5, 2.8)	<0.0001
High Glucose	MV	0.7 (0.5, 0.9)	0.003	0.5 (0.3, 0.6)	<0.0001	1.1 (0.8, 1.5)	0.47
	MV + SAT	1.0 (0.7, 1.4)	0.98	0.6 (0.5, 0.9)	0.009	1.4 (1.0, 1.9)	0.03
Diabetes	MV	1.6 (0.9, 2.6)	0.09	1.2 (0.7, 2.2)	0.47	0.5 (0.2, 1.0)	0.048
	MV + SAT	4.8 (2.6, 9.2)	<0.0001	2.6 (1.4, 4.9)	0.004	1.0 (0.5, 2.0)	0.91
Hypertension	MV	0.8 (0.6, 1.0)	0.08	0.4 (0.3, 0.5)	<0.0001	0.8 (0.6, 1.1)	0.13
	MV + SAT	1.5 (1.0, 2.1)	0.03	0.6 (0.4, 0.9)	0.01	1.1 (0.8, 1.6)	0.48
CVD	MV	1.2 (0.7, 2.0)	0.62	0.9 (0.5, 1.7)	0.76	1.3 (0.7, 2.3)	0.41
	MV + SAT	1.8 (0.9, 3.5)	0.07	1.2 (0.6, 2.5)	0.57	1.7 (0.9, 3.1)	0.11
Metabolic Syndrome	MV	0.6 (0.4, 0.8)	0.0002	0.3 (0.2, 0.4)	<0.0001	1.0 (0.8, 1.4)	0.86
	MV + SAT	2.5 (1.7, 3.6)	<0.0001	0.6 (0.4, 0.9)	0.02	2.3 (1.7, 3.2)	<0.0001
Hypertensive Treatment	MV	0.9 (0.7, 1.3)	0.75	0.5 (0.3, 0.8)	0.002	0.8 (0.5, 1.1)	0.20
	MV + SAT	1.9 (1.2, 2.8)	0.004	0.8 (0.5, 1.3)	0.31	1.1 (0.8, 1.7)	0.54
Lipid Treatment	MV	0.9 (0.6, 1.3)	0.54	0.6 (0.4, 0.9)	0.02	1.1 (0.8, 1.6)	0.62
	MV + SAT	1.2 (0.8, 1.8)	0.40	0.8 (0.5, 1.2)	0.21	1.3 (0.9, 1.9)	0.20

*Estimates give difference in odds of the dependent variable in each group as compared to the referent group (low SAT HU/low ratio). Multivariable (MV) model adjusts for age, current smoking status, alcohol use, physical activity and hormone replacement therapy (models in women only). MV+SAT model also adjusts for absolute volume of SAT.

Table 4: Multivariable Linear Regression for Selected CVD Risk Factors as Compared to the Referent Group (Low Density/Low Ratio).*

Dependent Variable		**High Density/ High Ratio**	**p-value**	**High Density/ Low Ratio**	**p-value**	**Low Density/ High Ratio**	**p-value**
Women							
Total Cholesterol (mg/dl)	MV	-7.0 (-12.2, -1.8)	0.008	-12.4 (-17.3, -7.5)	<0.0001	3.2 (-1.8, 8.2)	0.21
	MV + SAT	-4.8 (-10.2, 0.6)	0.08	-10.0 (-15.2, -4.8)	0.0002	4.0 (-1.1, 9.1)	0.12
HDL Cholesterol (mg/dl)	MV	-3.2 (-5.6, -0.8)	0.01	6.3 (4.1, 8.6)	<0.0001	-3.7 (-6.0, -1.3)	0.002
	MV + SAT	-6.7 (-9.1, -4.3)	<0.0001	2.5 (0.1, 4.8)	0.04	-5.0 (-7.2, -2.7)	<0.0001
Log Triglycerides (mg/dl)	MV	0.1 (-0.0, 0.1)	0.06	-0.3 (-0.3, -0.2)	<0.0001	0.2 (0.1, 0.2)	<0.0001
	MV + SAT	0.2 (0.1, 0.3)	<0.0001	-0.1 (-0.2, -0.1)	0.0003	0.2 (0.1, 0.3)	<0.0001
Fasting Glucose (mg/dl)	MV	4.8 (2.2, 7.4)	0.0004	-2.5 (-5.0, 0.0)	0.051	2.8 (0.3, 5.4)	0.03
	MV + SAT	8.9 (6.2, 11.5)	<0.0001	2.1 (-0.5, 4.6)	0.11	4.3 (1.9, 6.8)	0.0006
SBP (mmHg)	MV	0.4 (-2.0, 2.8)	0.75	-5.3 (-7.5, -3.0)	<0.0001	2.8 (0.5, 5.1)	0.02
	MV + SAT	3.8 (1.3, 6.2)	0.002	-1.5 (-3.8, 0.8)	0.21	4.0 (1.8, 6.3)	0.0005
DBP (mmHg)	MV	-0.4 (-1.7, 1.0)	0.57	-3.5 (-4.8, -2.3)	<0.0001	2.0 (0.7, 3.3)	0.003
	MV + SAT	1.4 (0.0, 2.8)	0.04	-1.5 (-2.9, -0.2)	0.02	2.6 (1.4, 3.9)	<0.0001
VAT/SAT Ratio	MV	0.28 (0.26, 0.31)	<0.0001	-0.01 (-0.03, 0.01)	0.26	0.23 (0.20, 0.25)	<0.0001
	MV + SAT	0.47 (0.44, 0.51)	<0.0001	-0.08 (-0.11, -0.04)	0.006	0.41 (0.38 0.45)	<0.0001
BMI (kg/m^2)	MV	-1.1 (-1.9, -0.2)	0.01	-3.3 (-4.1, -2.5)	<0.0001	0.1 (-0.8, 0.9)	0.88
	MV + SAT	3.3 (2.9, 3.7)	<0.0001	1.6 (1.2, 2.0)	<0.0001	1.7 (1.3, 2.1)	<0.0001
Waist Circumference	MV	-3.5 (-5.7, -1.3)	0.002	-9.8 (-11.8, -7.7)	<0.0001	1.1 (-1.0, 3.2)	0.30
	MV + SAT	7.7 (6.6, 8.7)	<0.0001	2.7 (1.7, 3.7)	<0.0001	5.4 (4.4, 6.4)	<0.0001
SAT (cm^3)	MV	-1231.0 (-1441.0, -1021.0)	<0.0001	-1368.4 (-1567, -1169.9)	<0.0001	-457.4 (-660.6, -254.2)	<0.0001
	MV + SAT	N/A	N/A	NA	N/A	N/A	N/A

VAT (cm^3)	MV	341.6 (239.5, 443.6)	<0.0001	-436.0 (-532.5, -339.5)	<0.0001	643.2 (544.4, 741.9)	<0.0001
	MV + SAT	819.5 (755.5, 883.5)	<0.0001	95.3 (33.8, 156.7)	0.002	820.8 (761.0, 880.6)	<0.0001
Subcutaneous HU (cm^3)	MV	6.9 (6.4, 7.5)	<0.0001	7.0 (6.5, 7.5)	<0.0001	0.5 (-0.1, 1.0)	0.10
	MV + SAT	5.8 (5.2, 6.3)	<0.0001	5.7 (5.2, 6.3)	<0.0001	0.0 (-0.5, 0.6)	0.90
Visceral HU (cm^3)	MV	-0.1 (-0.6, 0.5)	0.80	3.4 (2.9, 3.9)	<0.0001	-3.8 (-4.3, -3.3)	<0.0001
	MV + SAT	-1.6 (-2.1, -1.2)	<0.0001	1.7 (1.2, 2.1)	<0.0001	-4.4 (-4.8, -3.9)	<0.0001
Men							
Total Cholesterol (mg/dl)	MV	-1.9 (-6.2, 2.5)	0.40	-6.5 (-11.1, -1.8)	0.006	4.8 (0.1, 9.5)	0.05
	MV + SAT	-2.4 (-7.6, 2.7)	0.35	-6.9 (-12.0, -1.8)	0.008	4.5 (-0.5, 9.4)	0.08
HDL Cholesterol (mg/dl)	MV	-0.9 (-2.4, 0.6)	0.24	5.0 (3.4, 6.7)	<0.0001	-1.4 (-3.1, 0.2)	0.10
	MV + SAT	-4.8 (-6.5, -3.0)	<0.0001	2.1 (0.3, 3.8)	0.02	-3.5 (-5.2, -1.8)	<0.0001
Log Triglycerides (mg/dl)	MV	0.1 (-0.0, 0.1)	0.12	-0.3 (-0.3, -0.2)	<0.0001	0.1 (0.1, 0.2)	0.0004
	MV + SAT	0.2 (0.1, 0.3)	<0.0001	-0.1 (-0.2, -0.0)	0.002	0.2 (0.2, 0.3)	<0.0001
Fasting Glucose (mg/dl)	MV	3.0 (-0.1, 6.0)	0.06	0.1 (-3.2, 3.3)	0.97	-1.0 (-4.3, 2.3)	0.54
	MV + SAT	10.0 (6.5, 13.5)	<0.0001	5.5 (2.0, 9.0)	0.002	2.8 (-0.6, 6.2)	0.10
SBP (mmHg)	MV	-1.2 (-3.0, 0.6)	0.18	-4.2 (-6.1, -2.3)	<0.001	0.2 (-1.7, 2.1)	0.83
	MV + SAT	2.3 (0.3, 4.4)	0.03	-1.5 (-3.5, 0.5)	0.15	2.1 (0.2, 4.1)	0.03
DBP (mmHg)	MV	-1.6 (-2.7, 0.4)	0.008	-3.8 (-5.0, -2.5)	<0.0001	0.1 (-1.1, 1.4)	0.82
	MV + SAT	1.0 (-0.4, 2.3)	0.16	-1.8 (-3.1, -0.5)	0.007	1.5 (0.2, 2.8)	0.02
VAT/SAT Ratio	MV	0.58 (0.54, 0.61)	<0.0001	0.00 (-0.03, 0.04)	0.92	0.47 (0.43, 0.50)	<0.0001
	MV + SAT	0.47 (0.44, 0.51)	<0.0001	-0.08 (-0.11, -0.04)	<0.0001	0.41 (0.38, 0.45)	<0.0001
BMI (kg/m^2)	MV	-3.0 (-3.6, -2.4)	<0.0001	-3.1 (-3.7, -2.5)	<0.0001	-1.3 (-2.0, -0.7)	<0.0001
	MV + SAT	3.0 (2.7, 3.4)	<0.0001	1.5 (1.2, 1.9)	<0.0001	1.9 (1.6, 2.3)	<0.0001
Waist Circumference	MV	-9.5 (-10.9, -8.1)	<0.0001	-9.8 (-11.3, -8.3)	<0.0001	-4.1 (-5.6, -2.6)	<0.0001

	MV + SAT	6.2 (5.4, 6.9)	<0.0001	2.4 (1.7, 3.2)	<0.0001	4.5 (3.7, 5.2)	<0.0001
SAT (cm^3)	MV	-1681.9 (-1814.7, - 1549.1)	<0.0001	-1302.7 (-1443.7, -1161.7)	<0.001	-919.7 (-1063.1, -776.3)	<0.0001
	MV + SAT	N/A	N/A	N/A	N/A	N/A	N/A
VAT (cm^3)	MV	63.1 (-43.2, 169.4)	0.25	-769.7 (-882.5, -656.8)	<0.0001	685.5 (570.7, 800.3)	<0.0001
	MV + SAT	1019.3 (931.8, 1106.8)	<0.0001	-29.0 (-116.0, 57.9)	0.51	1208.4 (1123.8, 1293.0)	<0.0001
Subcutaneous HU (cm^3)	MV	6.4 (6.0, 6.8)	<0.0001	6.7 (6.2, 7.1)	<0.0001	0.7 (0.3, 1.2)	0.001
	MV + SAT	4.5 (4.0, 4.9)	<0.0001	5.2 (4.7, 5.6)	<0.0001	-0.3 (-0.7, 0.1)	0.17
Visceral HU (cm^3)	MV	1.2 (0.7, 1.6)	<0.0001	5.5 (5.0, 6.0)	<0.0001	-2.7 (-3.2, -2.2)	<0.0001
	MV + SAT	-1.1 (-1.6, -0.7)	<0.0001	3.7 (3.2, 4.2)	<0.0001	-4.02 (-4.5, -3.5)	<0.0001

*Estimates give difference in odds of the dependent variable in each group as compared to the referent group (low SAT HU/low ratio). Multivariable (MV) model adjusts for age, current smoking status, alcohol use, physical activity and hormone replacement therapy (models in women only). MV+SAT model also adjusts for absolute volume of SAT.