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Abdominal and Pericoronary Adipose Tissue Quantification with Computed Tomography and Their Relationship to Cardiovascular Risk Factors and Inflammatory Markers

Ph.D. Thesis

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Table of Content

DOI:10.14753/SE.2012.1663

DOI:10.14753/SE.2012.1663

DOI:10.14753/SE.2012.1663

Abreviations

ANOVA	Analysis of Variance
BMI	Body Mass Index
CT	Computed Tomography
CVD	Cardiovascular Disease
EAT	Epicardial Adipose Tissue
FFA	Free Fatty Acids
HDL	High Density Lipoprotein
hs-CRP	High Sensitivity C-reactive Protein
HU	Hounsfield Unit
ICAM-1	Intercellular Adhesion Molecule-1
ICC	Intra-class Correlation Coefficient
IL-6	Interleukin-6
IQR	Inter Quartile Range
LAD	Left Anterior Descendent Coronary Artery
LCx	Left Circumflex Coronary Artery
LM	Left Main Coronary Artery
Lp-PLA2	Lipoprotein Associated Phospholipase A2
MCP-1	Monocyte Chemoattractant Protein-1
MDCT	Multidetector-row Computed Tomography
MetS	Metabolic Syndrome
MRI	Magnetic Resonance Imaging
PAI-1	Plasminogen Activator Inhibitor
PCAT	Pericoronary Adipose Tissue
RCA	Right Coronary Artery
SAT	Subcutaneous Adipose Tissue
SD	Standard Deviation
SAA	Subcutaneous Adipose Tissue Area
SAV	Subcutaneous Adipose Tissue Volume
TNF-α	Tumor Necrosis Factor Alpha
VAT	Visceral Adipose Tissue
VAA	Visceral Adipose Tissue Area
VAV	Visceral Adipose Tissue Volume
WHO	World Health Organization
WL	Waistline

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1 Introduction

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in the industrialized countries (1). Improvements in CVD risk factor profiles have led to significant reductions in death from CVD over the past 50 years (2), but recent data suggest that the increasing prevalence of obesity may have slowed this rate of decline (3). The prevalence of obesity is beginning to exceed the prevalence of undernutrition and infectious diseases in several developing countries, which means that the full impact of the obesity epidemic has yet to be realized (4-8).

Furthermore, obesity increases the risk to develop less well-known complications, which include certain types of cancer, hepatic steatosis, gallbladder disease, respiratory complications (obstructive sleep apnea), endocrine abnormalities, obstetric complications, trauma to the weight-bearing joints, gout, cutaneous disease, proteinuria, increased hemoglobin concentration, and possibly immunologic impairment (9). Epidemiological studies and life-insurance data confirm that increasing degrees of overweight are important predictors of decreased longevity (10). In the Framingham Heart Study, the risk of death within 26 years increased by 1% for each extra 0.45 kilogram increase in weight between the ages of 30 years and 42 years, and by 2% between the ages of 50 years and 62 years (11).

In clinical practice and in epidemiological studies the body fatness is most commonly estimated by the body-mass index (BMI), which represents weight in kilograms divided by the square of the height in meters (12). The World Health Organization (WHO) expert committee proposed the classification of overweight and obesity which applies to all adult age groups (Table 1.) (13,14).

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Table 1 - Cut-off points proposed by a WHO expert committee for the classification of overweight

BMI ($kg.m^{-2}$)	WHO classification	Popular description
<18,5	Underweight	Thin
18,5-24,9	-	"normal", "acceptable"
25,0-29,9	Grade 1 overweight	Overweight
30,0-39,9	Grade 2 overweight	Obesity
≥40,0	Grade 3 overweight	Morbid obesity

The main assumption behind the BMI as a metric of adiposity is that most variation in weight for persons of the same height is due to fat mass. The graded classification of overweight using BMI values provides valuable information about increasing body fatness. Body mass index allows useful comparisons of weight within and between populations and the identification of individuals and groups at highest risk of morbidity and mortality. However, it is important to appreciate that, owing to differences in body proportions, BMI may not correspond to the same degree of fatness across different populations. Nor does it account for the wide variation in the nature of obesity between different individuals and populations (12).

Despite these shortcomings in the calculation the relationship between BMI and the incidence of several chronic conditions caused by excess fat is approximately linear for a range of BMI indexes less than 30 kg m^{-2}, but all risks are greatly increased for those subjects with a BMI above 29 kg m^{-2}, independent of gender (15,16).

1.1 Abdominal adipose tissue compartment

Despite all of the health hazards of obesity there are some very obese patients without any complications and with fairly normal metabolic risk profile. On the other hand, there are some moderately obese patients who develop multiple metabolic and atherogenic abnormalities. It was previously demonstrated that, for a given BMI or total amount of body fat, the subgroup of patients with excessive intra-abdominal, or visceral adipose

tissue depot is at substantially higher risk of developing insulin resistance and metabolic syndrome (17).

In the mid-twentieth century, Jean Vague, a French physician, made an important clinical observation that went largely unnoticed at the time. He described that body fat distribution, rather than excess body weight *per se*, was one of the key components associated with the presence of diabetes mellitus and CVD (18). Even at that time, he had suggested the concept that android obesity (upper body obesity) was associated with the risk of developing metabolic dysfunction conditions such as diabetes, CVD, and gout.

During the past two decades it became clear that, different ectopic fat compartments may be associated with differential metabolic risk (19). Several metabolic studies demonstrated that central type of obesity pose a greater risk for developing obesity-related disorders than BMI alone (20,21). In particular, the visceral adipose tissue (VAT) compartment may be a unique pathogenic fat depot (21-23). Visceral adipose tissue has been termed an endocrine organ, in part because it secretes adipocytokines and other vasoactive substances that can influence the risk of developing metabolic traits (23-26). It has been further emphasized by many metabolic investigations that excess visceral adiposity is a key feature of a phenomenon referred to as ectopic fat deposition, which has been connected to a plethora of metabolic dysfunctions (27). Available studies report relations of greater subcutaneous adipose tissue (SAT) and VAT with a higher prevalence of impaired fasting glucose (21,28), diabetes (21,23,29), insulin resistance (21,30,31), hypertension (32-34), atherogenic dyslipidemia (35-39), impaired fibrinolysis/increased risk of thrombosis and inflammation (40-42). It should be emphasized that these metabolic features, most commonly found in the viscerally obese patient, are often referred as the metabolic syndrome, which is linked to the development of cardiovascular disease (CVD). The metabolic syndrome of visceral obesity has been described as a "multiplex" additional modifiable CVD risk factor that - when added to traditional risk factors determines global "cardiometabolic risk" (43,44).

Inflammation is one of the important factors in the development of CVD and associated adverse clinical events (45). In the past decade, it became clear that chronic low-grade inflammation, such as is encountered in individuals with an excess of visceral/ectopic fat plays an important role in several cardiovascular disorders (46). In terms of its

proinflammatory and metabolic features, visceral adiposity is an emergent powerful but modifiable risk factor for CVD. Therefore, the precise and reproducible quantification of ectopic fat depots is important in order to further characterize the role of adipose tissue in the development of cardiovascular disorders.

Waist circumference measurement is widely used in clinical practice to assess abdominal obesity (47). The waistline (WL) correlates with measures of risk for coronary heart disease such as hypertension or blood lipid levels. The choice of cut-off points on the waist circumference continuum involves a trade-off between sensitivity and specificity similar to that for BMI. Gender-specific cut-off points for waist circumference may be of guidance in interpreting values for adults: proposed cut-off levels are shown in Table 2, with level 1 being intended to alert clinicians to potential risk, whereas level 2 should initiate therapeutic action (48). Waistline measurement is easily obtainable, however it is important to note that it is an imprecise measure of abdominal adiposity (49) because it is a function of both the SAT and VAT compartments. Therefore, assessment of VAT requires imaging with radiographic techniques such as computed tomography (CT) or magnetic resonance imaging (MRI).

Table 2 – Waistline predicts risk of metabolic complications

	Increased risk	**Substantially increased risk**
Men	≥94 cm	≥102 cm
Women	≥88 cm	≥88 cm

Gender-specific waistlines are presented as "increased risk" (level 1) and "substantially increased risk" (level 2) of metabolic complications associated with obesity in Caucasian population.

Current imaging studies evaluating abdominal fat depots are limited to small, referral-based samples often enriched for adiposity-related traits (33,35-37,50-52). Furthermore, study samples have often been limited to either women or men, precluding the study of sex differences (32,33,35-38,52,53). Some studies have focused on Japanese Americans or Southeast Asians (29,32,36,39,53,54), ethnic groups with more visceral fat than expected for a given overall BMI (55).

Multidetector-row CT permits highly reproducible volumetric measurements of both SAT and VAT (56). In addition, this initial observation suggested that volumetric fat measurements - as opposed to previous studies using single-slice methodology - can accurately characterize the heterogeneity of abdominal fat distribution between individuals and the differences in fat distribution with age and between women and men. Furthermore, no data is available in a community-based sample of women and men free of CVD across the spectrum of BMI whether the volume of SAT (subcutaneous adipose tissue volume, SAV) and VAT (visceral adipose tissue volume, VAV) are associated with metabolic risk factors and markers of inflammation cross-sectionally. It is not fully understood whether VAT is more strongly associated with metabolic risk factors than is SAT.

1.2 Pericoronary adipose tissue compartment

Recently, an influence of thoracic adipose tissue on the development of coronary artery disease (CAD) has been suggested, as it has been shown that pericardial fat is closely associated with cardiovascular risk factors and cardiovascular disease (57,58). Epicardial adipose tissue (EAT) covers 70-100% of the cardiac circumference as a layer of adipose tissue between the myocardium and the visceral pericardium (59). Notably, EAT and intra-abdominal fat originate from the same visceral white preadipocytes during embryogenesis and both are rich source of bioactive molecules (60). EAT secretes several pro- and anti-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukine-6 (IL-6), free fatty acids (FFA), plasminogen activator inhibitor-1 (PAI-1), and adipocytokines such as adiponectin (61-63). It has been suggested that adipocytokines produced by fat surrounding the coronary arteries might amplify vascular inflammation and generate a pro-atherogenic processes in the vessel wall from ‘outside-to-inside’ (64,65). Elevated pericardial fat volumes may lead to a mismatch in the production of pro- and anti-inflammatory mediators (66). The portion of EAT which directly surrounds the coronary arteries is termed as pericoronary adipose tissue (PCAT). The pericoronary adipose tissue compartment may have a key role in the development of

coronary artery disease through a paracrine effect that causes amplification of inflammation, plaque instability, and neovascularization (24).

The precise and reproducible quantification of this rather small, pericoronary visceral fat compartment is crucial for the understanding of its role in coronary artery atherogenesis. The most commonly used techniques for PCAT quantification in cardiac computed tomography (CT) datasets are based on thickness and area measurements on a limited number of axial image slices (65,67-69). Recent studies reported a moderate to poor reproducibility of the PCAT thickness and area measurements, with a reported coefficient of variation between 12.0% - 23.4% and intra-class correlation coefficient of 0.76 (95% CI 0.50 – 0.88) (65,67,68). Moreover, these 2-dimensional quantification techniques do not reflect the inhomogeneous distribution of PCAT along the atrioventricular and interventricular grooves due to the limitations posed by the single CT cross-sectional assessment.

There is no three-dimensional, threshold based quantification method available for PCAT assessment. The few available studies have used PCAT thickness measurement in single slices and reported contradictory results regarding the relationship between the severity of coronary atherosclerosis and PCAT quantity (67,68). Furthermore, it is not fully understood whether PCAT is a marker of overall adiposity or an independent pathogenic fat depot.

We hypothesize that precise and reproducible three-dimensional quantification of PCAT could provide insights into the pathophysiological role of this distinct adipose tissue depot in coronary atherosclerotic plaque development.

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2 Aims

2.1 Planimetric and volumetric adipose tissue quantification methods

We sought to assess the intra- and inter-observer reproducibility of MDCT based volumetric quantification of subcutaneous and visceral abdominal adipose tissue. Furthermore, our aim was to investigate the differences between the relative amounts of visceral and subcutaneous abdominal tissue quantity as assessed by volumetric and planimetric methods. In addition, we investigated the relation of MDCT based measurements to anthropometric measures of obesity such as BMI, sagital diameter (SD) and waistline (WL).

2.2 Abdominal adipose tissue volumes and metabolic risk factors

We aim to assess whether the volume of SAT and VAT are associated with metabolic risk factors in a community-based sample free of CVD. Furthermore, we sought to determine whether sophisticated volumetric imaging methods of SAT and VAT provide information about metabolic risk other than that offered by classic anthropometric measures such as BMI and WL.

2.3 Abdominal adipose tissue volumes and markers of inflammation and oxidative stress

We aim to investigate the association of abdominal fat compartment volumes with a panel of systemic inflammatory markers. Furthermore, we sought to assess whether volumetric measurements of SAT and VAT explained additional interindividual variability in biomarker concentrations above that accounted for by the simple clinical anthropometric measures of obesity BMI and WL.

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2.4 Novel volumetric pericoronary adipose tissue quantification

Cardiac computed tomography (CT) allows for simultaneous assessment of PCAT volume and coronary artery plaque. Volumetric quantification of PCAT in patients with and without CAD in conjunction with high and low hs-CRP levels could provide insights into the pathophysiological role of this distinct adipose tissue depot and its local effect on coronary atherosclerosis. Thus, we aimed to 1) assess the feasibility and reproducibility of a novel threshold-based method of PCAT volume quantification using cardiac CT, 2) determine the relationship of PCAT volume to the presence of coronary atherosclerotic plaque on patient, vessel and subsegment basis. We have examined the surrounding PCAT volume in groups of patients with presence of coronary plaque and high hs-CRP and no plaque and low hs-CRP levels, furthermore we have investigated a third intermediate group with no plaque and high hs-CRP level.

3 Methods

3.1 Study population

3.1.1 Framingham Heart Study

Participants for this study were drawn from the Framingham Heart Study Multidetector Computed Tomography Study, a population-based substudy of the community-based Framingham Heart Study Offspring and Third-Generation Study cohorts. Beginning in 1948, 5209 men and women 28 to 62 years of age were enrolled in the original cohort of the Framingham Heart Study. The offspring and spouses of the offspring of the original cohort were enrolled in the Offspring Study starting in 1971. Selection criteria and the original study design have been described elsewhere (70,71). Beginning in 2002, 4095 Third Generation Study participants, who had at least 1 parent in the offspring cohort, were enrolled in the Framingham Heart Study and underwent standard clinic

examinations. The standard clinic examination included a physician interview, a physical examination, and laboratory tests. For the present study, the study sample consisted of Offspring and Third Generation Study participants who were part of the multidetector-row CT substudy.

Between June 2002 and April 2005, 3529 participants (2111 third generation, 1418 offspring participants) underwent multidetector-row CT assessment of coronary and aortic calcium. Inclusion in this study was weighted toward participants from larger Framingham Heart Study families and those who resided in the greater New England area. Overall, 755 families were included in our analysis. Men had to be ≥35 years of age; women had to be ≥40 years of age and not pregnant; and all participants had to weigh <160 kilograms. Of the participants, 433 (222 offspring and 211 third generation) were imaged as participants in an ancillary study using an identical imaging protocol, the National Heart, Lung, and Blood's Family Heart Study (72). Of the total 3529 subjects imaged, 3394 had interpretable CT measures; of those, 3329 had both SAT and VAT measured; of those, 3124 of them were free of CVD; of those, 3102 attended a contemporaneous examination; and of those, 3001 had a complete covariate profile. Thus, the overall sample size for analysis is 3001.

The biomarkers and inflammatory substances were assessed in the offspring participants with technically interpretable CT scans (n=1377), attendance at the seventh examination cycle (1998-2001; n=1355), complete covariate information (n=1253), and measurement of at least one inflammatory marker, resulting in a total sample size of 1250 participants. Sample size varied slightly for individual markers; TNF-α and isoprostane measurements were only available on a subset (n=920 and 1010, respectively). The CT scan was performed an average of 4.2 years after the seventh examination cycle (when covariates and inflammatory markers were measured).

The study was approved by the institutional review boards of the Boston University Medical Center and Massachusetts General Hospital, Harvard Medical School. All subjects provided written informed consent.

3.1.2 ROMICAT study

From May 2005 to May 2007 consecutive subjects were prospectively enrolled as part of the ROMICAT (Rule Out Myocardial Infarction using Computer Assisted Tomography) trial. Details of the study have been previously reported (73). From the 368 ROMICAT patients, we included 51 patients in this age- and gender-matched 1:1:1 case-control design. Patients were stratified into 3 groups based on presence of coronary atherosclerosis and hs-CRP levels. Group 1 included patients with presence of coronary plaque and hs-CRP >2.0 mg/L; intermediate group (Group 2) included patients with no plaque and hs-CRP >2.0 mg/L, Group 3 included patients with no plaque and hs-CRP <1.0 mg/L. The hs-CRP cutoff points were selected according to data from recent clinical trials and scientific statements (74-76). We included only patients with right coronary artery (RCA) dominance or co-dominance to avoid potential bias due to small RCA in coronary systems with left dominance.

3.2 Abdominal adipose tissue assessment

3.2.1 MDCT scan protocol for abdominal adipose tissue quantification

All subjects underwent CT scanning in a supine position using an eight-slice MDCT (LightSpeed Ultra, General Electric, Milwaukee, WI, USA). Twenty-five contiguous 5 mm thick slices (120 kVp, 400 mA, gantry rotation time 500 ms, table feed 3:1) were acquired covering 125 mm above the level of S1. The raw data were reconstructed using a 55 cm field of view. The effective radiation exposure was 2.7 mSv.

3.2.2 Measurements of abdominal adipose tissue areas and volumes

We measured the subcutaneous and visceral adipose tissue areas (SAA and SAA) and volumes (SAV and SAV) as well as the waistline (WL) and sagital diameter (SD) using a

dedicated offline workstation (Aquarius 3D Workstation, TeraRecon Inc., San Mateo, CA, USA). The SAA (cm^2) and SAA (cm^2) as well the WL (cm) and SD (cm) were measured using a single slice (5 mm thickness) at the umbilical level (77,78). In CT absolute Hounsfield units (HU) of pixels correspond directly to the tissue property. Thus, we applied predefined image display setting to determine the visceral and subcutaneous adipose tissue areas using a window width of -195 to -45 HU and a window center of -120 HU to identify pixels containing adipose tissue (79,80). In order to separate visceral from subcutaneous adipose tissue the abdominal muscular wall separating the two compartments was manually traced.

The SAV and VAV were measured across the total imaging volume and were calculated in cm^3. We applied a semi-automatic segmentation technique using the same image display settings as for the area measurements. The abdominal muscular wall separating the two adipose tissue compartments was manually traced in four sections of the imaging volume representing the quartiles of the scanning range (1st, 9th, 17th, and 25th slice). The segmentation of the entire scanning volume was performed automatically interpolating the information of the manually defined traces. If necessary, manual adjustments were made throughout the scan volume. The average time for image analysis was 5 minutes per subject.

3.2.3 Measurements of sagital diameter and waistline

The sagital abdominal diameter (SD) and the waistline (WL) were measured at the level of the umbilicus. The SD (cm) was defined as the shortest distance between the mid-anterior wall of the abdomen and the mid-posterior wall. The WL (cm) was directly measured by tracing the circumference of the abdominal skin.

3.2.4 Reproducibility

The reproducibility study sample represents a random subset of 100 Caucasian subjects (age range: 37 – 83 years; 49% female) drawn from the offspring cohort, who underwent MDCT scanning (n = 1418). The random sample was taken to ensure approximately

equal number of men and women, and an approximately equal number of participants in each of the age groups of 35-44, 45-54, 55-64, 65-74 and 75-84 years, were represented (approximately 10 per age group per sex).

Two observers performed an independent analysis of all datasets in random order to assess for inter-observer variability. One reader repeated the analysis one week later to assess for intra-observer variability.

3.2.5 Risk factor and covariate assessment

Risk factors and covariates were measured at the contemporaneous examination. BMI was measured at each index examination, and standing WL, obtained at the level of the umbilicus, were measured by trained technicians following written protocols. Fasting plasma glucose, total and high-density lipoprotein (HDL) cholesterol, and triglycerides were measured on fasting morning samples. Diabetes was defined as a fasting plasma glucose level ≥126 mg/dL at a Framingham examination or treatment with either insulin or a hypoglycemic agent. Hypertension was defined as systolic blood pressure of at least 140 mmHg or diastolic blood pressure of at least 90 mmHg or current antihypertensive treatment. Prevalent cardiovascular disease was defined at the seventh examination cycle as coronary heart disease, stroke, heart failure, or intermittent claudication as described previously (81). Chronic aspirin use was defined as self-reported aspirin use three or more times/week. Participants were considered current smokers if they had smoked at least 1 cigarette per day for the previous year. Assessed through a series of physician-administered questions, alcohol use was dichotomized on the basis of consumption of >14 drinks per week (in men) or 7 drinks per week (in women). Physical activity, determined by questionnaire, was represented as the weighted sum of the proportion of a typical day spent sleeping and performing sedentary, slight, moderate, or heavy physical activities (82). Post-menopausal status was defined as cessation of menses for at least one year. Impaired fasting glucose was defined as a fasting plasma glucose level of 100 to 125 mg/dL among those not treated for diabetes. Metabolic syndrome (MetS) was defined from modified Adult Treatment Panel criteria (83).

3.2.6 Biomarker assessment

Biomarkers were measured on fasting morning samples collected at the participant's visit during the seventh examination cycle (1998-2001) as previously described (84). Samples were stored at -80ºC and thawed at the time of analysis (with exception of urine, see below). Serum CRP serum was measured by high-sensitivity assay [(Dade Behring BN100 nephelometer; mean intra-assay CV 3.2%]. Fibrinogen was measured in duplicate from citrated plasma using Clauss method (Diagnostica Stago Reagents, CV 2.1%). Other markers were measured in duplicate by enzyme-linked immunosorbent assay commercially available kits [R&D Systems: intercellular adhesion molecule-1 (ICAM-1), interleukin-6, monocyte chemoattractant protein-1 (MCP-1), P-selectin, tumor necrosis factor receptor 2 (TNFR2), high-sensitivity TNF-alpha (TNFα); Bender MedSystems: CD40 ligand; GlaxoSmithKline: lipoprotein associated phospholipase A2 (Lp-PLA2) activity and mass; OXIS: myeloperoxidase; ALPCO Diagnostics: osteoprotegerin)]. Mean intra-assay CVs were as follows: plasma specimens: CD40 ligand 4.4%, fibrinogen 1.1%, Lp-PLA2 activity 7.0%, Lp-PLA2 mass 5%, osteoprotegerin 3.7%, P-selectin 3.0%, TNFα 6.6%, TNFR2 2.2%; serum specimens: ICAM-1 3.7%, interleukin-6 3.1%, MCP1 3.8%, myeloperoxidase 3.0%, osteoprotegerin 3.7%. Isoprostane (8epi-PGF2α) production was measured in duplicate from urine samples using a commercially available enzyme-linked immunosorbent assay (Cayman, Ann Arbor, MI; CV 9.6± 6.8), and indexed to urinary creatinine concentrations (Abbot Spectrum CCX; CV 2-4%), expressed as ng/mmol, as previously described (85).

3.3 Pericoronary adipose tissue assessment

3.3.1 MDCT scan protocol for pericoronary adipose tissue quantification

Contrast-enhanced gated CT imaging was performed using a standard coronary artery 64-slice MDCT (Sensation 64, Siemens Medical Solutions, Forchheim, Germany) imaging protocol that included the administration of 0.6 mg sublingual nitroglycerin in the absence of contraindications and 5-20 mg of intravenous metoprolol if the baseline heart

rate was above 60 beats per minute. Images were acquired during a single inspiratory breath hold in spiral mode with 330 ms rotation time, 32 x 0.6 mm collimation, tube voltage of 120 kVp, maximum effective tube current-time product of 850 mAs, and tube modulation when possible. On average, 80 mL of iodinated contrast agent (Iodixanol 320 g/cm^3, Visipaque, General Electrics Healthcare, Princeton, NJ), followed by 40 mL saline solution was injected at a rate of 5 mL/s. A test bolus scan with 20 mL iodinated contrast followed by 40 mL saline at 5ml/s was used to calculate the beginning of image acquisition according to contrast agent transit time. Trans-axial data sets were reconstructed at 65% phase, with an image matrix of 512x512 pixels, a slice thickness of 0.75 mm, and an increment of 0.4 mm. All image analyses were performed offline on dedicated cardiac workstations.

3.3.2 Pericoronary adipose tissue volumetric assessment

We started the PCAT quantification at the ostium of the left main (LM)/left anterior descending coronary artery (LAD), left circumflex artery (LCx), and right coronary artery (RCA). The method of threshold-based volumetric PCAT assessment is based on a modified application of software for volumetric assessment of coronary atherosclerotic plaques (Vitrea 2, Version 3.9.0.1, Vital Images Inc, Plymouth, MN and SUREPlaque, Toshiba Medical Systems, Tustin, CA). Manual tracing was used to circle the region containing PCAT in cross-sectional images perpendicular to the vessel centerline in every 5 mm. The exact pericoronary fat volume within the manually traced region was calculated by the software using Hounsfield unit (HU) based thresholds (Figure 1).

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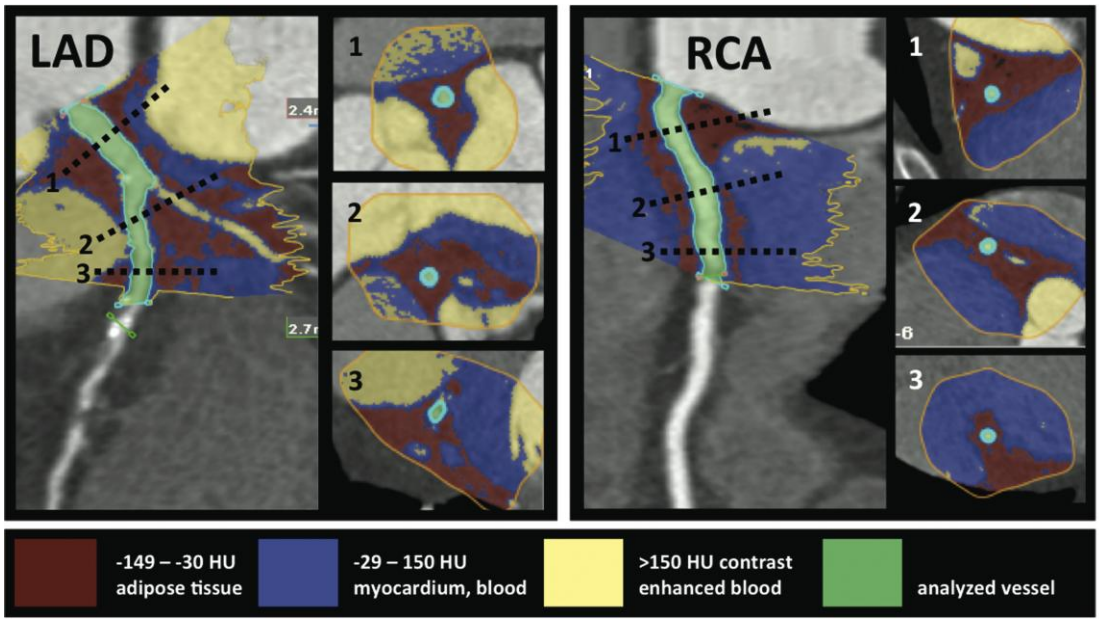

Figure 1 - Threshold based volumetric pericoronary adipose tissue quantification (left panel: LAD, right panel: RCA). The inserted cross-sections correspond to levels marked with dotted lines on the LAD and RCA. The manual tracing (yellow line) of the region of interest is performed on muliplanar-reformatted images perpendicular to the vessel centerline. The voxels within the predefined Hounsfield unit range are summarized, and the adipose tissue volume is calculated automatically. The red color indicates fat containing voxels. The blue color indicates the vessel wall, myocardium, and the non-enhanced blood pool. The yellow color indicates the contrast enhanced blood filled lumen and cavities. The green color indicates the analyzed vessel.

Voxels with values between the minimum setting of the SUREPlaque tool (-149HU) and the widely used upper threshold (-30HU) (86) were determined to represent adipose tissue, and the total PCAT volume was calculated by summing these voxels along the course of each coronary artery. We recorded PCAT volumes in 5 mm increments, and summed PCAT along the measured vessel lengths. Voxels representing air, myocardium, or contrast enhanced blood pool in the selected region were excluded by the HU cutoffs. Two independent readers who were blinded to the coronary plaque and hs-CRP results performed the PCAT measurements. We have analyzed the proximal part of the coronary vessels since this vessel segment has a better image quality on coronary CT angiography as compared to the distal segments (87). Furthermore, this is the most relevant vessel

portion clinically, since the proximal segments contain the majority of culprit lesions in patients with acute coronary syndromes (88). Observer 1 performed measurements around the proximal 40 mm of the coronaries in all 51 patients once. For inter-observer and intra-observer reproducibility, observer 2 performed the measurements in 20 randomly selected patients, and Observer 1 repeated this process in the same 20 patients 1 month later. A total of 153 vessels and 1224 coronary artery subsegments were evaluated across the three patient groups.

3.3.3 Coronary artery plaque assessment

The coronary plaque assessment was performed by one experienced CT reader who was blinded to the PCAT volume and hs-CRP results. To determine the exact anatomic location of coronary plaques, the first 40 mm of the LM/LAD, LCx, and the RCA were evaluated using axial images, multiplanar reconstructions, thin-slab maximum intensity projections, and curved multiplanar reformatted images. The presence of coronary plaque was evaluated in 5 mm subsegments of each vessel: 0-5 mm, 5-10 mm, 10-15 mm, 15-20 mm, 20-25 mm, 25-30 mm, 30-35 mm, and 35-40 mm positions from the ostium.

3.3.4 Hs-CRP assessment

Blood samples from the peripheral vein were collected in an EDTA-coated tube within one hour prior to the coronary CT angiography scan. The samples were immediately centrifuged with the plasma aliquoted and stored at -80°C until analysis. Concentrations of hs-CRP were measured nephelometrically on a BN II analyzer (Dade-Behring, Marburg, Germany). Inter-assay coefficients of variation were <5% for nephelometry. Hs-CRP levels were defined as high if >2.0 mg/L and low if <1.0 mg/L, as described above.

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3.4 Statistical analysis

3.4.1 Abdominal fat quantification reproducibility study: Statistical considerations

Two experienced observers performed an analysis of all 100 datasets in random order to assess for inter-observer variability, blinded to the readings by the other observer. One reader repeated the analysis one week later to assess for intra-observer variability. Inter- and intra-observer reproducibility was assessed using the intra-class correlation coefficient (ICC) (89). A value close to 1 indicates excellent agreement between the two readings. In addition, the significance of the mean difference between the two readings was assessed using the paired t-test. Similar analysis was used to compare single- and volumetric- measurements for the first reading of the primary reader. The age and sex effect on the difference between single- and volumetric-subjects were assessed individually using one-way analysis of variance. A p-value < 0.05 was considered to indicate statistical significance.

3.4.2 Abdominal adipose tissue and metabolic risk factors: Statistical considerations

SAV and VAV were normally distributed. Sex-specific age-adjusted Pearson correlation coefficients were used to assess simple correlations between SAV and VAV and metabolic risk factors. Multivariable linear and logistic regression was used to assess the significance of covariate-adjusted cross-sectional relations between continuous and dichotomous metabolic risk factors and SAT and VAT. For continuous risk factors, the covariate-adjusted average change in risk factor per 1–standard deviation (SD) increase in adipose tissue was estimated; for dichotomous risk factors, the change in odds of the risk factor prevalence per 1-SD increase in adipose tissue was estimated. All models were sex specific to account for the strong sex interactions observed. Covariates in all models included age, smoking (3-level variable: current/former/never smoker), physical activity, alcohol use (dichotomized at >14 drinks per week in men or >7 drinks per week in women), menopausal status, and hormone replacement therapy. In addition, lipid treatment, hypertension treatment, and diabetes treatment were included as covariates in

models for HDL cholesterol, log triglycerides, systolic and diastolic blood pressures, and fasting plasma glucose, respectively. R^2 values were computed for continuous models and c statistics were computed for dichotomous models to assess the relative contribution of SAT and VAT to explain the outcomes (risk factors). For each risk factor, tests for the significance of the difference between the SAT and VAT regression coefficients were carried out within a multivariate standardized regression (in which variables were first standardized to a mean of 0 and an SD of 1) to assess the relative importance of each adipose tissue measurement in predicting the risk factor. To assess the incremental utility of adding VAT to models that contain BMI or WL, the above multivariate analyses were repeated for VAT with BMI and WL added as covariates in the multivariate regression models. Similar models were not examined for SAT because models with SAT alone did not yield higher R^2 or c statistics than models that included BMI and WL alone. As a secondary analysis, the above multivariate regressions were rerun using the general estimating equation linear and logistic regression (90) account for correlations among related individuals (siblings) in the study sample. SAS version 8.0 was used to perform all computations; a 2-tailed value of $P<0.05$ was considered significant (90).

3.4.3 Abdominal adipose tissue and markers of inflammation: Statistical considerations

All biomarkers were log-transformed due to their skewed distributions. Analyses described below were sex-pooled, except for CRP analyses, which were performed for each sex separately as we observed a significant sex-interaction.

Age- and sex-adjusted Pearson partial correlation coefficients were used to assess correlations between SAT and VAT and biomarkers. In our primary analysis multivariable linear regression models were constructed for each biomarker (dependent variable) versus each of SAT and VAT separately adjusted for age, sex, cigarette smoking (current, former, never smoker), chronic aspirin use, alcohol consumption, menopausal status, hormone replacement therapy, and physical activity index. R^2 were computed to assess the relative contribution of SAT and VAT towards explaining the variance in each biomarker. For each marker that was significantly correlated with both SAT and VAT, we used multivariable regressions to assess the significance of the marker

relationship with SAT in the presence of VAT and vice-versa.

We report the magnitude of the association of SAT and VAT with each biomarker concentration by calculating regression coefficients quantifying the estimated change in log transformed biomarker per standard deviation increase in SAT and VAT separately, and then transforming back to original biomarker units. SAT and VAT were first standardized to a mean of 0 and standard deviation of 1. Multivariable linear regression containing both SAT and VAT as regressor variables were used to compare the SAT and VAT beta-coefficients for each marker. We then examined the residual effect size of SAT and VAT as it related to each biomarker in models containing the above-mentioned risk factors and BMI and WL. Secondarily, we examined the significance of BMI and WL in these multivariable-adjusted models containing SAT or VAT.

We performed several additional secondary analyses. First, we tested for the presence of interactions of VAT and SAT with sex, age (above/below the median age of 60 years), smoking (current, former, never), and obesity (yes/no) in the above multivariable models (without BMI and WL as independent variables). Second, we performed analyses excluding individuals with diabetes or prevalent cardiovascular disease as determined at exam seven. Third, we conducted analyses also adjusting for systolic blood pressure, diastolic blood pressure, hypertension treatment, lipid treatment, total/high density lipoprotein cholesterol ratio, triglycerides, diabetes, and prevalent cardiovascular disease. Finally, we conducted exploratory analyses excluding individuals with CRP>10 mg/L at examination seven to account for acute inflammatory states.

To account for multiple testing, we limited our definition of statistical significance to a two-tailed $p<0.05$ for primary analyses (for each individual marker), and $p<0.01$ for all secondary analyses. SAS version 8.0 was used to perform all computations (90).

3.4.4 Pericoronary adipose tissue quantification: Statistical considerations

Continuous variables are reported as mean ± standard deviation (SD) or median and interquartile range (IQR), as appropriate. Discrete variables are given in frequency and percentiles. To compare the differences in characteristics between the three groups, we used analysis of variance (ANOVA) or Kruskal-Wallis test for continuous variables as

appropriate and Fisher's Exact test for categorical variables. For reproducibility of PCAT volumes, we used the intraclass correlation coefficient (ICC) for inter-observer and intra-observer agreement and paired t-test for determining the significance of the mean absolute and relative differences. In addition, inter-observer measures were assessed using the Pearson's correlation coefficient and a Bland-Altman graph. On a per-patient basis, the differences in PCAT volumes between the 3 groups were determined with ANOVA, and post-hoc two group comparisons were performed with Wilcoxon rank sum tests. We used generalized linear regression analysis to adjust for covariates with p-value <0.10 in univariate analyses, which included body mass index (BMI), hypertension, and hyperlipidemia. On a per-vessel basis, the differences in PCAT volumes between the 3 groups were assessed using ANOVA. On a per-subsegment basis, we compared the surrounding PCAT volume in the 1224 subsegments with plaque and no plaque using the Wilcoxon rank sum test and confirmed the results by using a mixed model with restricted maximum likelihood estimation to account for within-subject correlation. A two-tailed p-value of <0.05 was considered significant. All analyses were performed using the SAS software (Version 9.2, SAS Institute Inc) and SPSS 16.0 (Chicago, Illinois).

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4 Results

4.1.1 The reproducibility study

The mean SAV was 2929.8 ± 1260.0 cm^3 (range of 501.0 – 6695.0) and the mean VAV was 2031.6 ± 1013.7 cm3 (range of 288.0 – 4731.0). The mean SAA was 543.5 ± 252.4 cm^2 and the mean SAA was 325.9 ± 162.3 cm^2. The mean WL was 100.0 ± 12.3 cm (range of 74.9 – 131.3) and the mean SD was 24.2 ± 4.0 cm (range of 15.9 – 35.9).

4.1.2 Intra-observer variability

The intra-observer reproducibility was excellent for SAV and VAV (ICC=0.99; Figure 2a). The mean absolute and relative intra-observer differences were small and non-significant for both measurements (SAV: -0.6 ± 6.1 cm^3, p=0.29; VAV: 0.7 ± 6.0 cm^3; p=0.26).

The mean absolute difference was 0.1 ± 0.6 cm (p=0.09) for WL measurements and -0.01 ± 0.2 cm (p=0.68) for SD measurements (Table 3). Both WL and SD measurements were highly correlated (ICC: 0.99).

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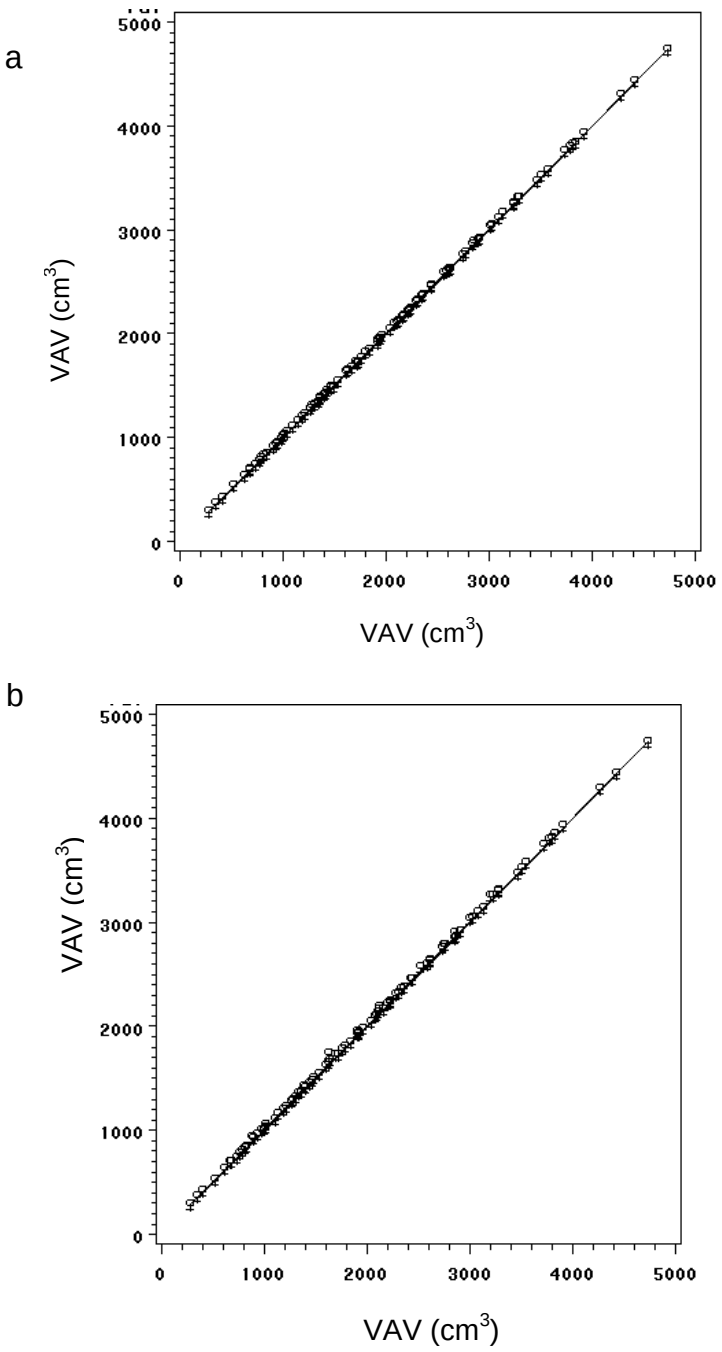

Figure 2 - a) Intra- and b) Inter-reader correlation of volumetric measurements of abdominal adipose tissue. Absolute mean intra-observer differences: 0.68 ± 6.0 cm^3; ICC=0.99 (b) Absolute mean inter-observer differences: 9.9 ± 14.8 cm^3; ICC=0.99.

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4.1.3 Inter-observer variability

The mean absolute inter-observer differences were extremely small and both measurements were highly correlated (SAV: -9.1 ± 12.0 cm3, ICC=0.99, and VAV: 9.9 ± 14.8 cm3; ICC=0.99 (Figure 2b)). The relative difference between observers was small and non-significant -0.34% ± 0.52% for SAV and 0.59% ± 0.93% for VAV (p=n.s.).
The mean WL was 100.0 ± 12.3 cm (range of 74.9 – 131.3) with a mean absolute difference of -0.1 ± 0.8 cm and a mean relative difference of -0.08% ± 0.84% between the two observers (ICC=0.99). The mean SD was 24.2 ± 4.0 cm (range of 15.9 – 35.9) with a mean absolute difference of -0.2 ± 0.4 cm and a mean relative difference of -0.73% ± 1.82% (ICC=0.99); (Table 3).

Table 3 - Inter- and intra-observer correlation.

	Inter-observer Variability			Intra-observer Variability		
	Mean Actual Difference	Percentage Difference	ICC	Mean Actual Difference	Percentage Difference	ICC
SAV	-9.1±12.0 cm^3	-0.34%±0.52%	0.99	-0.6±6.1 cm^3	0.34%±0.52%	0.99
VAV	9.9±14.8 cm^3	0.59%±0.93%	0.99	0.68±6.0 cm^3	0.59%±0.93%	0.99
WL	-0.1±0.8 cm	-0.08%±0.84%.	0.99	0.1±0.6 cm	0.08%±0.84%	0.99
SD	-0.2±0.4 cm	-0.73%±1.82%	0.99	-0.01±0.2 cm	0.73%±1.82%	0.99

Abbreviations: ICC, Intra Class Correlation; SAV, Subcutaneous Adipose Tissue Volume; VAV, Visceral Adipose Tissue Volume; WL, Waist Circumference; SD, Sagital Diameter.

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4.1.4 Ratio of subcutaneous and visceral adipose tissue volumes

The mean SAA/VAA ratio (2.0 ± 1.2; range: 0.5 – 6.7) was significantly greater than the mean SAV/VAV ratio (1.7 ± 0.9; range: 0.4 – 5.3); ($p<0.001$) (Figure 3). This difference was more evident in 22 subjects with a SAA/VAA ratio ≥ 2.5 (mean difference: -0.9 ± 0.7; $p<0.001$). An example for the difference between planimetric and volumetric based assessment of adipose tissue distribution is given in Figure 4.

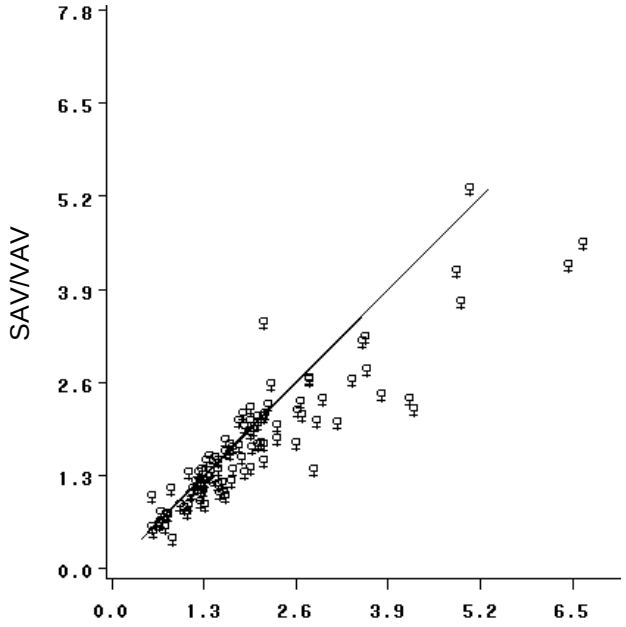

Figure 3 - Association between volume and area based measurements of the ratio between subcutaneous and visceral adipose tissue. The mean SAV/VAV ratio was significantly different from the mean SAA/VAA ratio (1.7 ± 0.9 vs. 2.0 ± 1.2; respectively) with a relative difference of 11.1% ($p<0.001$), ICC: 0.84 SAV: Subcutaneous adipose tissue volume, VAV: Visceral adipose tissue volume, SAA: Subcutaneous adipose tissue area, VAA: Visceral adipose tissue area

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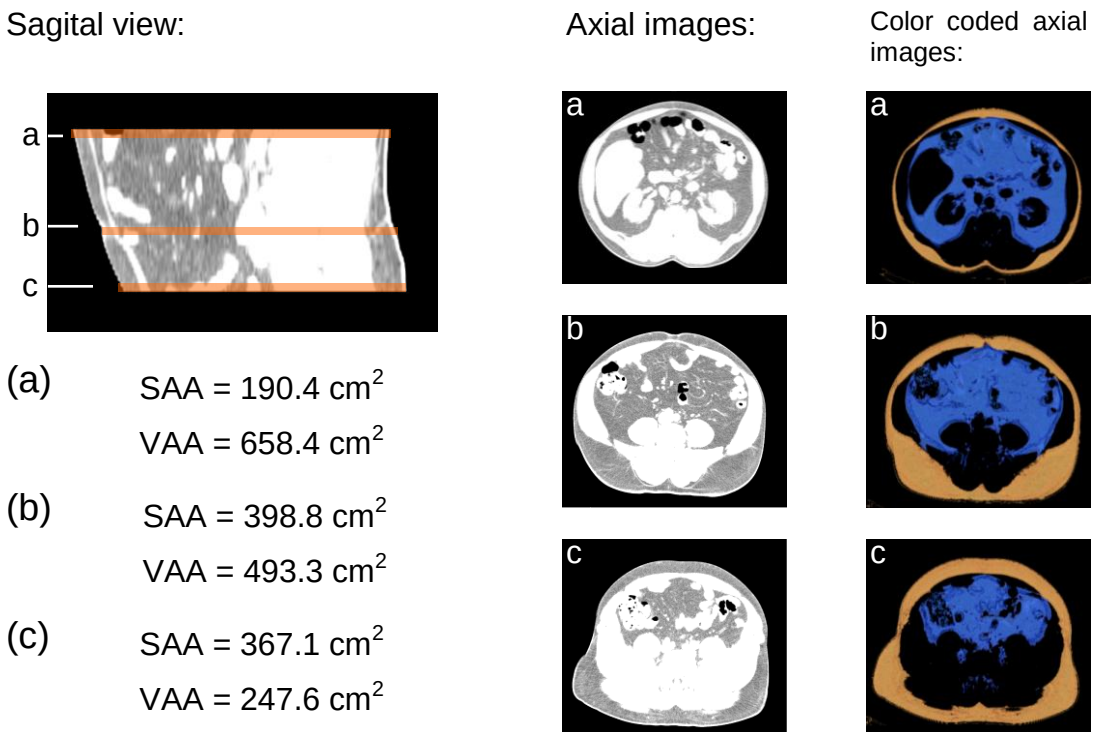

Figure 4 - Example of the variable distribution of visceral and subcutaneous abdominal adipose tissue in a 56 year old men. Area based measurements performed at the level of the umbilicus (b); level L4/L5 (a), and S1 level (c). Ratios between subcutaneous and visceral adipose tissue vary considerably in this subject. The color coded axial images are the result of semi automatic segmentation (blue= visceral adipose tissue; orange=subcutaneous adipose tissue).

4.1.5 Relation of volumetric based adipose tissue measurements to WL, SD, and BMI

Both SAV and VAV were highly correlated to anthropometric measurements (for SAV: r=0.83, 0.73, 0.75 and for VAV: r=0.76, 0.85, 0.70; for WL, SD, and BMI; respectively, all $p<0.0001$). In contrast, the ratio of SAV to SAV was only weakly inversely associated with SD (r=-0.32, p=0.01) and not correlated with WL (r=-0.14, p=0.14) or BMI (r=-0.17, p=0.09). As expected, anthropometric measurements were strongly correlated with each other (BMI vs. WL r=0.87, $p<0.0001$; BMI vs. SD r=0.84, $p<0.001$; and SD vs. WL r=0.94, $p<0.0001$).

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4.1.6 Abdominal adipose tissue distribution by age and sex

In order to determine whether volumetric measurements reflect differences in abdominal adipose tissue distribution related to age, we stratified our population to above (n=51) and below (n=49) the mean age (59.9 ± 12.9 years). The mean SAV/VAV ratio was significantly higher in subjects <60 years of age as compared to subjects > 60 years (1.9 ± 1.0 vs. 1.5 ± 0.7; $p<0.001$). In addition, we examined for possible differences between men and women, and we found that men had significantly lower SAV/VAV ratios than women independent of age (1.2 ± 0.5 vs. 2.2 ± 0.9 for men vs. women; respectively ($p<0.001$)).

4.2 The abdominal adipose tissue depots and metabolic risk factors

Overall, 1452 women and 1549 men were available for analysis. The mean age was 50 years (Table 4); approximately one quarter of the sample was hypertensive, 5% had diabetes, and approximately one third had MetS. Approximately half of the women were postmenopausal.

In the analysis regarding the metabolic risk factors the third generation's participants were included as well. The mean SAT volume among the offspring and the third gen participants was 3071±1444 cm^3 in women and 2603±1187 cm^3 in men. The mean VAT volume in women was 1306±807 cm^3 and in men was 2159±967 cm^3.

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Table 4 - Clinical characteristics of study participants free of clinical CVD who underwent MDCT assessment of SAT and VAT volumes

	Women (n=1452)	Men (n=1549)
Age, y	51±9	49±10
BMI, kg/m^2	26.7±5.4	28.3±4.4
WL, cm	92±14	103±11
Triglycerides,* mg/dL	92 (65, 134)	113 (76, 171)
HDL cholesterol, mg/dL	62±17	46±12
Total cholesterol, mg/dL	197±36	196±34
Systolic blood pressure, mmHg	119±17	123±14
Diastolic blood pressure, mmHg	73±9	78±9
Hypertension, %	24	28
Fasting plasma glucose, mg/dL	95±16	101±20
Impaired fasting glucose,† %	18	38
Diabetes mellitus, %	4.2	6.1
MetS, %	25	35
Smoking, %		
Current	12	13
Former	42	34
Never	46	53
Postmenopausal, %	48	...
Hormone replacement therapy, %	23	...
Alcohol use, ‡ %	15	16
SAT, cm^3	3071±1444	2603±1187
VAT, cm^3	1306±807	2159±967

Data are presented as mean±SD when appropriate.
*Median (25th, 75th percentiles).
†Fasting plasma glucose of 100 to 125 mg/dL; percentage is based on those without diabetes.
‡Defined as >14 drinks per week (men) or >7 drinks per week (women).

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4.2.1 Correlations with SAT and VAT

Correlations of SAT and VAT with metabolic risk factors are shown in Table 4. SAT was positively correlated with age in women (r=0.13, P<0.001) but not men, and VAT was positively correlated with age in both sexes (r=0.36 in women and men, P<0.001). SAT and VAT were highly correlated, with an age-adjusted correlation coefficient between SAT and VAT of 0.71 (P<0.0001) in women and 0.58 (P<0.0001) in men. Both BMI and WL were strongly correlated with SAT and VAT after adjustment for age (Table 5). All risk factors were highly correlated with both SAT and VAT, except for serum total cholesterol with SAT in men and physical activity index with VAT in men.

Table 5 - Age-adjusted Pearson Correlation coefficients between metabolic risk factors and SAT and VAT volumes

	Women		Men	
	SAT	VAT	SAT	VAT
Age	0.13†	0.36†	0.03†	0.36†
BMI	0.88†	0.75†	0.83†	0.71†
WL	0.87†	0.78†	0.88†	0.73†
Log triglycerides	0.31†	0.46†	0.18†	0.37†
HDL cholesterol	–0.25†	–0.35†	–0.17†	–0.33†
Total cholesterol	0.11†	0.15†	0.02	0.08*
Systolic blood pressure	0.26†	0.30†	0.18†	0.24†
Diastolic blood pressure	0.26†	0.28†	0.21†	0.27†
Blood glucose	0.23†	0.34†	0.12†	0.19†
Physical activity index	–0.14†	–0.09*	–0.08*	–0.03

*p<0.01; †p<0.001

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4.2.2 Multivariable-adjusted regressions with SAT, VAT, and metabolic risk factors

Results of multivariable-adjusted general linear regression analyses for SAT and VAT for both continuous and dichotomous metabolic risk factors are shown in Table 5. In women, per 1-SD increase in SAT, systolic blood pressure increased on average 3.9±0.4 mmHg (±1 SE), whereas VAT was 4.8±0.4 mmHg higher. For systolic blood pressure in women, the difference between the magnitude of effect of the SAT versus VAT was not significant (P=0.10; (Table 5.). In men, the magnitude of the association of the average systolic blood pressure increase per 1-SD increase in VAT was larger than for SAT (3.3 versus 2.3 mmHg, respectively; P=0.01 for difference in the regression coefficients between SAT and VAT). Similar results were obtained for diastolic blood pressure.

In women and men, the associations of both SAT and VAT with continuous measures of metabolic risk factors were highly significant. For fasting plasma glucose, the effect of VAT was stronger than that of SAT (P<0.0001 for difference in women, P=0.001 in men). Strong and significant results for log triglycerides and HDL cholesterol followed similar patterns (Table 6).

Highly significant associations with SAT and VAT also were noted for dichotomous risk factor variables. Among women and men, both SAT and VAT were associated with an increased odds of hypertension (Table 5). In women, the odds ratio of hypertension per 1-SD increase in VAT (odds ratio, 2.1) was stronger than that for SAT (odds ratio, 1.7; P=0.001 for difference between SAT and VAT); similar differences were noted for men. Similar highly significant differences also were noted for impaired fasting glucose, diabetes, and MetS and are presented in Table 5.

The magnitude of association between VAT and all risk factors examined was consistently greater for women than for men (Table 5). Weaker sex differences were observed for SAT.

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Table 6 - Gender-specific multivariable-adjusted* regressions for SAT and VAT with continuous metabolic risk factors (top) and dichotomous risk factors (bottom)

	Women				Men				
	MV-adjusted residual effect size	p for either SAT or VAT	p for SAT vs VAT	Residual effect size after MV/BMI/ WL adjustment	MV-adjusted residual effect size	p for either SAT or VAT	p for SAT vs VAT	Residual effect size after MV/BMI/ WL adjustment	p for sex interaction
SBP									
SAT	3.9±0.4	<0.0001		...	2.3±0.3	<0.0001		...	0.01
VAT	4.8±0.4	<0.0001	0.10	2.5±0.7	3.3±0.4	<0.0001	0.01	1.8±0.05	<0.0001
DBP									
SAT	2.2±0.2	<0.0001		...	1.9±0.2	<0.0001		...	0.77
VAT	2.6±0.3	<0.0001	0.33	1.4±0.4	2.6±0.2	<0.0001	0.008	1.5±0.3	0.01
FPG									
SAT	3.4±0.3	<0.0001		...	1.6±0.4	0.0002		...	0.03
VAT	4.8±0.4	<0.0001	<0.0001	3.4±0.6	3.1±0.5	<0.0001	0.001	1.8±0.7	<0.0001
Log TG									
SAT	0.14±0.01	<0.0001		...	0.10±0.01	0.003		...	0.16
VAT	0.23±0.01	<0.0001	<0.0001	0.19±0.02	0.22±0.01	<0.0001	<0.0001	0.22±0.02	0.0002
HDL									
SAT	–3.9±0.4	<0.0001		...	–2.0±0.3	<0.0001		...	0.006
VAT	–5.9±0.4	<0.0001	<0.0001	–4.5±0.7	–4.5±0.3	<0.0001	<0.0001	–3.8±0.5	<0.0001
HTN									
SAT	1.7 (1.5–2.0)	<0.0001		...	1.5 (1.4–1.7)	<0.0001		...	0.89
VAT	2.1 (1.8–2.4)	<0.0001	<0.0001	1.6 (1.3–2.0)	1.9 (1.6–2.1)	<0.0001	0.006	1.6 (1.3–1.9)	0.01
IFG									
SAT	2.0 (1.7–2.3)	<0.0001		...	1.5 (1.3–1.7)	<0.0001		...	0.04
VAT	2.5 (2.1–2.9)	<0.0001	0.001	2.1 (1.7–2.6)	1.8 (1.6–2.0)	<0.0001	0.005	1.5 (1.2–1.8)	<0.0001
DM									
SAT	1.6 (1.2–2.0)	0.007		...	1.6 (1.3–1.9)	<0.0001		...	0.27
VAT	2.1 (1.6–2.6)	<0.0001	0.0003	1.9 (1.3–2.7)	1.6 (1.3–2.0)	<0.0001	0.91	0.9 (0.7–1.3)	0.03
MetS									
SAT	3.0 (2.6–3.5)	<0.0001		...	2.5 (2.2–2.8)	<0.0001		...	0.77
VAT	4.7 (3.9–5.7)	<0.0001	<0.0001	1.9 (1.3–2.7)	4.2 (3.5–5.0)	<0.0001	<0.0001	2.6 (2.1–3.2)	0.002

MV indicates multivariable; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TG, triglycerides; HTN, hypertension; IFG, impaired fasting glucose; and DM, diastolic mellitus. Data presented include effect size (the average change in risk factor□SE) per 1 SD in adipose tissue for continuous data, and the change in odds of the condition per 1 SD of adipose tissue with 95% CIs for dichotomous data.

*Adjusted for age, smoking, alcohol use, physical activity, and menopausal status (women only), hormone replacement therapy (women only); for blood pressure, FPG, HDL cholesterol, and log triglycerides, an additional covariate of treatment for HTN, diabetes, or lipid disorders, respectively, was included.

†For SAT or VAT in the model. ‡For SAT vs VAT difference.

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4.2.3 Residual effect of VAT in multivariable models that contain BMI and WL

To address whether radiographic imaging of abdominal adipose tissue explains variation in metabolic risk factors over and above the contribution of BMI and WL, we examined the residual effect size of each metabolic risk factor from multivariable models that additionally contained BMI and WL. Because models with BMI and WL routinely yielded higher R^2 or c statistic than models with SAT (Table 7A and 7B) the addition of all 3 variables into one model was not pursued. For example, in women, SAT plus covariates were associated with 21% of the variation in log triglycerides (R^2=0.21), VAT plus covariates were associated with 30% of the variation in log triglycerides, and both BMI and WL plus the covariates were associated with 26% of the variation in triglycerides. Models with VAT, BMI, and WL demonstrated significant additional contribution of VAT for all variables except diabetes in men. Statistically significant residual effect sizes for VAT were observed for all metabolic risk factors except diabetes in men (Table 7).

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Table 7 A - R^2 (for continuous data) for multivariate models of individual metabolic risk factors before and after adding VAT to the models.

	Women		Men	
	R^2	p-value for VAT in models with BMI/WL	R^2	p-value for VAT in models with BMI/WL
Systolic blood pressure				
MV covariates alone*	0.22		0.13	
+SAT	0.27		0.16	
+VAT	0.28		0.18	
+BMI+WL	0.28		0.18	
+BMI+WL+VAT	0.29	0.0003	0.18	0.0005
Diastolic blood pressure				
MV covariates alone*	0.05		0.03	
+SAT	0.10		0.07	
+VAT	0.11		0.10	
+BMI+WL	0.11		0.09	
+BMI+WL+VAT	0.12	0.0007	0.11	<0.0001
Fasting plasma glucose				
MV covariates alone*	0.39		0.27	
+SAT	0.43		0.28	
+VAT	0.46		0.29	
+BMI+WL	0.45		0.30	
+BMI+WL+VAT	0.46	<0.0001	0.30	<0.01
Log Triglycerides				
MV Covariates alone*	0.13		0.01	
+SAT	0.21		0.04	
+VAT	0.30		0.14	
+BMI+WL	0.26		0.09	
+BMI+WL+VAT	0.30	<0.0001	0.14	<0.0001
HDL Cholesterol				
MV Covariates alone*	0.09		0.07	
+SAT	0.14		0.10	
+VAT	0.19		0.18	
+BMI+WL	0.17		0.15	
+BMI+WL+VAT	0.19	<0.0001	0.19	<0.0001

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Table 7 B - c-statistics (for dichotomous data) for multivariate models of individual metabolic risk factors before and after adding VAT to the models.

	Women		Men	
	c-stat.	p-value for VAT in models with BMI/WL	c-stat.	p-value for VAT in models with BMI/WL
Impaired fasting glucose				
MV covariates alone*	0.67		0.61	
+SAT	0.74		0.66	
+VAT	0.78		0.68	
+BMI+WL	0.76		0.68	
+BMI+WL+VAT	0.78	<0.0001	0.69	<0.0001
Diabetes				
MV covariates alone**	0.77		0.72	
+SAT	0.78		0.75	
+VAT	0.81		0.76	
+BMI+WL	0.81		0.79	
+BMI+WL+VAT	0.82	0.0006	0.79	0.68
Hypertension				
MV covariates alone**	0.75		0.71	
+SAT	0.78		0.73	
+VAT	0.80		0.75	
+BMI+WL	0.80		0.74	
+BMI+WL+VAT	0.80	<0.0001	0.75	<0.0001
Metabolic Syndrome				
MV Covariates alone**	0.71		0.62	
+SAT	0.83		0.76	
+VAT	0.88		0.82	
+BMI***	0.86		0.82	
+BMI+WL+VAT	0.89	<0.0001	0.82	<0.0001

*Adjusted for age, smoking, alcohol use, physical activity, and menopausal status (women only), hormone replacement therapy (women only); for blood pressure, FPG, and HDL cholesterol, and log triglycerides, an additional covariate of treatment for HTN, diabetes, or lipid disorders, was included.

**Adjusted for age, smoking, alcohol use, physical activity, and menopausal status (women only), hormone replacement therapy (women only)

***For metabolic syndrome, models were only adjusted for BMI because of the inclusion of WL criteria in the metabolic syndrome definition

Abbreviations: MV=multivariable; SAT=subcutaneous adipose tissue, VAT=visceral adipose tissue, SD=standard deviation, SBP=systolic blood pressure, DBP=diastolic blood pressure, FPG=fasting plasma glucose, TG=triglycerices, HDL=high density lipoprotein, HTN=hypertension, IFG=impaired fasting glucose, DM=diabetes, BMI=body mass index. WL=waist circumference

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4.2.4 Risk factor distribution based on quartiles of VAT

Because VAT adds to risk factor variation above and beyond BMI and WL, we assessed the impact of stratifying individuals by VAT quartile within clinically defined categories of BMI (normal weight, BMI <25 kg/m^2; overweight, BMI of 25 to 29.9 kg/m^2; and obese, BMI ≥30 kg/m^2). Thirty-three percent of the sample was normal weight, 41% was overweight, and 26% was obese. Among normal-weight, overweight, and obese individuals, there was a highly statistically significant stepwise linear increase in the prevalence of the MetS across quartiles of VAT in both women and men (Figure 5) after adjustment for age and BMI; similar relations were noted for additional risk factors, including hypertension and impaired fasting glucose.

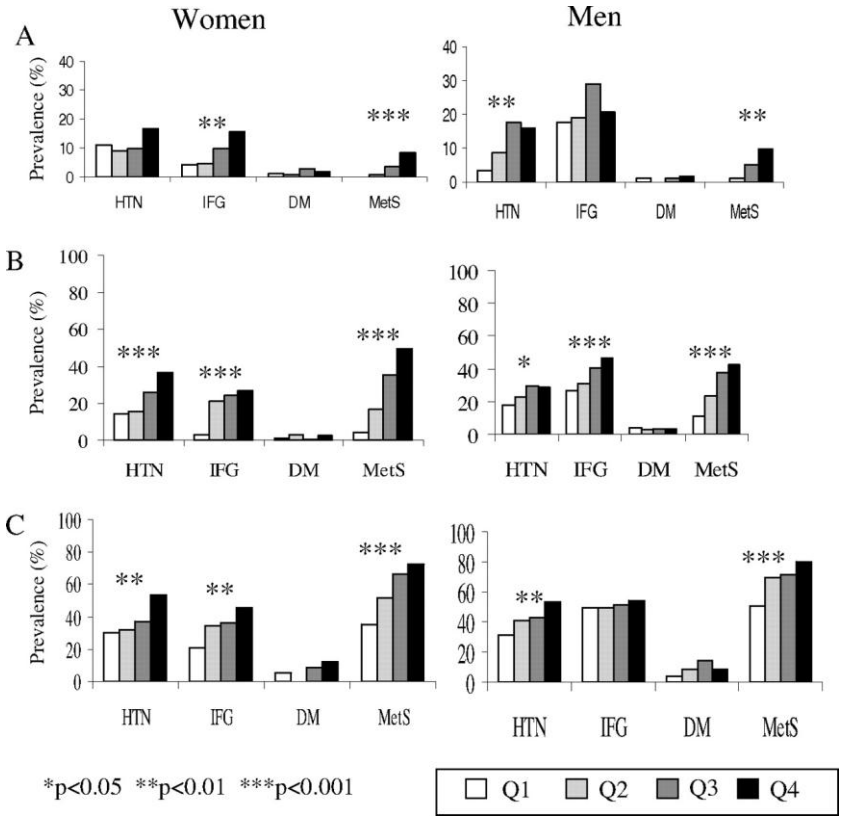

Figure 5. Prevalence of hypertension (HTN), impaired fasting glucose (IFG), diabetes (DM), and MetS among normal-weight (A), overweight (B), and obese (C) individuals. Probability values represent those for linear trend across VAT quartiles and are adjusted for age and BMI.

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4.3 Abdominal adipose tissue depots and the markers of inflammation and oxidative stress

The participants' clinical, multidetector-row CT, and biomarker characteristics are shown in table 8A and 8B. The mean age of the 1250 individuals (52% women) was 60±9 years. Mean SAT was 3023±1329 cm^3, and mean VAT was 2126±1112 cm^3.

Table 8 A - Clinical and CT characteristics of Participants

Charactersitic	
Age, y	60±9
Women, %	52
BMI, $kg/^{m2}$	28.3±5.1
Waist circumference, cm	100±14
Smoking, current:former:never, %	10:52:38
Aspirin 3 times/wk, %	31
Alcohol intake, 14 drinks/wk (men) or 7 drinks/wk (women), %	16
Postmenopausal, % (women)	83
Hormone replacement therapy, % (women)	36
Physical activity index	38±6
Hypertension treatment, %	30
Total cholesterol/HDL ratio	4.1±1.3
Triglycerides, mg/dL	138±96
Diabetes mellitus, %	10
Prevalent cardiovascular disease, %	12
CT fat measures	
SAT, cm^3	
Sex-pooled	3023±1329
Women	3320±1424
Men	2699±1133
VAT, cm^3	
Sex-pooled	2126±1112
Women	1645±870
Men	2652±1110

Values are mean±SD or percent. HDL indicates high-density lipoprotein.

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Table 8 B - Biomarkers of participants

Biomarkers	
CRP, mg/L	
Women	2.5 (1.1 to 6.0)
Men	1.7 (0.9 to 3.7)
CD40 ligand, plasma, ng/mL	1.3 (0.6 to 4.1)
Fibrinogen, mg/dL	368 (326 to 414)
ICAM-1, ng/mL	239 (210 to 274)
IL-6, pg/mL	2.6 (1.7 to 4.1)
Isoprostanes, pg/mL	1137 (541 to 1986)
Lp-PLA2 activity, nmol · mL^{-1} · min^{-1}	142 (120 to 166)
Lp-PLA2 mass, ng/mL	283 (231 to 355)
MCP-1, pg/mL	306 (248 to 380)
Myeloperoxidase, ng/mL	40.4 (28.3 to 60.9)
Osteoprotegerin, pmol/L	5.2 (4.3 to 6.1)
P-selectin, ng/mL	36 (28 to 45)
TNF-α, pg/mL	1.2 (0.9 to 1.6)
TNF receptor-2, pg/mL	1955 (1671 to 2336)

Values are median (25th to 75th percentile). ICAM, intercellular adhesion molecule; Lp-PLA2, lipoprotein-associated phospholipase A2; MCP, monocyte chemoattractant-1; and TNF, tumor necrosis factor.

4.3.1 Correlations with SAT and VAT

SAT and VAT were positively and similarly correlated with most circulating inflammatory biomarkers (Table 9). CD40 ligand, lipoprotein-associated phospholipase A2 (Lp-PLA2), osteoprotegerin, and tumor necrosis factor (TNF)-α were not correlated with either SAT or VAT. BMI and WL were correlated with the same biomarkers as SAT and VAT and were additionally correlated with TNF-α.

Table 9 - Pearson correlation coefficients between log-transformed biomarkers and SAT, VAT, BMI, and WL (age- and sex-adjusted).

Marker	No.	SAT	VAT	BMI	WL
CRP*					
Women	646	0.45†	0.47†	0.52†	0.51†
Men	596	0.30†	0.33†	0.38†	0.37†
CD40 ligand, plasma	1241	–0.02	–0.05	–0.04	–0.04
Fibrinogen	1243	0.27†	0.22†	0.29†	0.27†
ICAM-1	1241	0.12†	0.12†	0.16†	0.16†
IL-6	1241	0.23†	0.23†	0.27†	0.25†
Isoprostanes	1010	0.13†	0.22†	0.18†	0.20†
Lp-PLA2 activity	1240	–0.02	0.02	0.01	0.01
Lp-PLA2 mass	1240	–0.04	–0.02	–0.04	–0.04
MCP-1	1226	0.07‡	0.13†	0.09‡	0.11†
Myeloperoxidase	1208	0.10†	0.06§	0.10†	0.10†
Osteoprotegerin	1240	0.01	0.03	0.04	0.06§
P-selectin	1243	0.09‡	0.12†	0.11†	0.14†
TNF-α	920	0.05	0.06	0.09‡	0.09‡
TNF receptor-2	1214	0.17†	0.12†	0.21†	0.19†

*CRP is a sex-specific correlation. †p<0.001, ‡p<0.01, §p<0.05.

4.3.2 Multivariable-adjusted regressions with SAT and VAT

In multivariable models, CRP, fibrinogen, intercellular adhesion molecule-1 (ICAM-1), IL-6, isoprostanes, monocyte chemoattractant-1 (MCP-1), P-selectin, and TNF receptor-2 remained associated with both SAT and VAT; myeloperoxidase was significantly associated with SAT and had a borderline association with VAT (Table 10). With the exception of CRP (sex interaction P=0.02 for SAT and P<0.0001 for VAT), there was no evidence of significant effect modification by sex on the association of SAT or VAT with other biomarkers. In women, for a 1-SD increase in SAT, estimated CRP was 1.7 mg/L higher on average, whereas for a 1-SD increase in VAT, CRP was 1.8 mg/L higher. In contrast, the association in men was less strong: For a 1-SD increase in SAT and VAT, estimated CRP was 0.6 and 0.7 mg/L higher, respectively. For most markers, the estimated increase in concentrations per 1 SD of SAT was comparable to and not statistically significantly different from that of VAT (Table 9), with 2 exceptions. For

isoprostanes, the magnitude of the estimated association with VAT was almost double that of SAT (Table 9; *P*=0.002 for difference in effect between SAT versus VAT). Although less striking, we also observed differences in the magnitude of the SAT versus VAT association with MCP-1 (Table 10; *P*=0.04 for SAT versus VAT comparison).

Table 10 - Multivariable-adjusted linear regression models of relation of SAT or VAT to biomarkers: R^2 and effect size of SAT or VAT, before and after adjustment for BMI and WL

	Multivariable model‡ plus SAT or VAT			Multivariable model‡ plus BMI/WL plus SAT or VAT		
	Model R^2	Increase in Marker§ per 1 SD of SAT or VAT	P*	Model R^2	Increase in Marker§ per 1 SD of SAT or VAT	P†
CRP, mg/L						
Women						
SAT	0.28	1.7 (1.4, 2.0)	<0.0001	0.34	0.11 (-0.21, 0.49)	0.51
VAT	0.29	1.8 (1.4, 2.1)	<0.0001	0.36	0.57 (0.25, 0.92)	0.0003
Men						
SAT	0.18	0.6 (0.4, 0.8)	<0.0001	0.23	0.02 (-0.18, 0.25)	0.84
VAT	0.19	0.7 (0.5, 0.9)	<0.0001	0.24	0.26 (0.1, 0.5)	0.006
CD40 ligand, ng/mL						
SAT	0.03	-0.05 (-0.13, 0.05)	0.33	0.03	0.03 (-0.12, 0.21)	0.69
VAT	0.03	-0.08 (-0.17, 0.02)	0.12	0.03	-0.05 (-0.18, 0.09)	0.44
Fibrinogen, mg/dL						
SAT	0.19	19 (15, 23)	<0.0001	0.20	8.0 (1.7, 14.3)	0.01
VAT	0.16	17 (13, 21)	<0.0001	0.20	3.4 (-2.0, 8.9)	0.22
ICAM-1, ng/mL						
SAT	0.11	7.4 (4.4, 10.5)	<0.0001	0.12	-1.3 (-6.4, 3.8)	0.61
VAT	0.11	7.3 (4.0, 10.7)	<0.0001	0.12	0.17 (-4.23, 4.67)	0.94
IL-6, pg/mL						
SAT	0.12	0.5 (0.3, 0.6)	<0.0001	0.14	0.12 (-0.05, 0.30)	0.17
VAT	0.12	0.5 (0.4, 0.6)	<0.0001	0.14	0.20 (0.05, 0.36)	0.01
Isoprostanes, pg/mL						
SAT‖	0.07	160 (83, 243)	<0.0001	0.09	-78 (-182, 37)	0.18
VAT	0.10	313 (218, 414)	<0.0001	0.10	223 (103, 355)	0.0002

Lp-PLA2 activity, nmol · mL^{-1} · min^{-1}						
SAT	0.22	0.4 (-1.4, 2.3)	0.65	0.23	-2.5 (-5.5, 0.6)	0.11
VAT	0.23	1.9 (-0.2, 4.0)	0.07	0.23	1.3 (-1.5, 4.2)	0.36
Lp-PLA2 mass, ng/mL						
SAT	0.04	-1.2 (-6.3, 4.1)	0.66	0.04	-1.1 (-9.8, 7.9)	0.81
VAT	0.04	0.1 (-5.7, 6.0)	0.97	0.04	1.6 (-6.3, 9.7)	0.70
MCP-1, pg/mL						
SAT[‖]	0.07	7.8 (1.7, 14.0)	0.01	0.07	-3.4 (-13.5, 7.1)	0.53
VAT	0.08	15.0 (8.2, 22.0)	<0.0001	0.08	12.5 (3.3, 21.9)	0.008
Myeloperoxidase, ng/mL						
SAT	0.05	2.6 (1.2, 4.0)	0.0002	0.05	1.6 (-0.7, 4.1)	0.20
VAT	0.04	1.5 (0.0, 3.0)	0.05	0.05	-0.7 (-2.6, 1.3)	0.50
Osteoprotegerin, pmol/L						
SAT	0.20	0.02 (-0.06, 0.10)	0.62	0.21	-0.13 (-0.26, 0.01)	0.06
VAT	0.20	0.05 (-0.04, 0.14)	0.25	0.20	-0.01 (-0.13, 0.11)	0.82
P-selectin, ng/mL						
SAT	0.06	1.3 (0.5, 2.0)	0.001	0.07	-0.6 (-1.8, 0.7)	0.37
VAT	0.07	1.9 (1.0, 2.7)	<0.0001	0.07	0.9 (-0.2, 2.1)	0.11
TNF-, pg/mL						
SAT	0.04	0.03 (0.00, 0.07)	0.08	0.04	-0.03 (-0.10, 0.03)	0.31
VAT	0.04	0.04 (0.00, 0.08)	0.06	0.04	0.00 (-0.06, 0.05)	0.92
TNF-α receptor-2, pg/mL						
SAT	0.16	97 (66, 128)	<0.0001	0.17	12.9 (-37.7, 64.7)	0.62
VAT	0.14	74 (40, 108)	<0.0001	0.17	-18.4 (-61.4, 25.6)	0.41

R^2=percentage of variance in the dependent variable that is explained by the independent variable(s). *P for SAT or VAT; [†]P for SAT or VAT in models with BMI/WL. [‡]Adjusted for sex, age, smoking, aspirin, alcohol intake, menopausal-status and hormone replacement therapy (women only), and physical activity index. [§]Average expected increase in biomarker concentration from the median biomarker concentration (95% CI). [‖]Comparison of model R2 for SAT vs VAT for each biomarker was only significant for isoprostanes (P=0.002) and MCP-1 (P=0.04).

4.3.3 Multivariable-adjusted regressions with both SAT and VAT in models

If SAT and VAT were included in the same multivariable-adjusted model, both SAT and VAT remained significant correlates of CRP, fibrinogen, and IL-6. Only VAT remained significantly associated with isoprostanes, MCP-1, and P-selectin, whereas only SAT remained significantly associated with ICAM-1, myeloperoxidase, and TNF receptor-2.

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4.3.4 Addition of SAT and VAT to multivariable models that included BMI and WL

To assess whether CT-based measures of abdominal fat compartments added to the amount of marker variability explained by models that already included BMI and WL, we added SAT and VAT separately to models that included BMI and WL (Table 9). In models that also adjusted for BMI and WL, SAT remained associated with fibrinogen only (P=0.01), whereas VAT remained significantly associated with CRP, IL-6, isoprostanes, and MCP-1. In women, after adjustment for BMI and WL, the additional estimated increase in CRP was 0.57 mg/L per 1 SD of VAT, whereas in men, the estimate was only half this magnitude.

4.3.5 Secondary analyses

We hypothesized that some previously described correlates of inflammatory markers, including systolic and diastolic blood pressure, lipid treatment, total cholesterol/HDL ratio, triglycerides, diabetes mellitus, and cardiovascular disease, might serve as intermediate mechanisms linking SAT, VAT, and inflammation. If these covariates were added to the models, results were not altered materially. Similarly, the exclusion of participants with cardiovascular disease (n=151), diabetes mellitus (n=129), or CRP concentrations >10 mg/L (n=94) did not substantively alter the present findings. Overall, we found evidence for a statistically significant but small degree of effect modification of age on the association between SAT and fibrinogen and between VAT and CRP. Additionally, we detected an effect modification of smoking on the association between SAT and 3 markers (CRP, IL-6, and isoprostanes). Current smoking essentially eliminated the association between SAT and both IL-6 and isoprostanes. Interactions with obesity were not significant for any marker (Table 11).

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Table 11 - Significant interactions* with SAT or VAT and markers in adjusted models†

		Increase in marker‡ per 1 SD of SAT/VAT	P-value for interaction
CRP	SAT + smoking		
	Current	0.9 (0.2,1.7)	0.005
	Former	1.2 (0.8,1.4)	
	Never	1.1 (0.9,1.5)	
	SAT + sex		
	Women	1.7 (1.4,2.0)	0.02
	Men	0.6 (0.4,0.8)	
	VAT + sex		
	Women	1.8 (1.4,2.1)	<0.0001
	Men	0.7 (0.5,0.9)	
	VAT + Age		
	<60 years old	1.2 (1.0,1.4)	0.002
	≥60 years old	1.4 (1.0,1.7)	
IL-6	SAT + smoking		
	Current	0.1 (-0.3,0.5)	0.003
	Former	0.5 (0.4,0.7)	
	Never	0.5 (0.3,0.7)	
Isoprostanes	SAT+ smoking		
	Current	-205 (-496,153)	0.005
	Former	164 (55,283)	
	Never	213 (98,340)	
Fibrinogen	SAT + Age		
	<60 years old	24.5 (19.5,29.5)	0.0002
	≥60 years old	11.7 (6.2,17.4)	

*Interactions tested: sex, age (<60), smoking (current, former, never), obesity †Adjusted for sex, age, smoking, aspirin, alcohol intake, menopausal-status and hormone replacement (women only), and physical activity index.

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To further investigate the relation between increasing VAT relative to SAT with inflammation and oxidative stress, we compared concentrations of CRP and isoprostanes divided on the basis of sex-specific SAT and VAT tertiles (Figure 6). CRP was associated with increasing SAT and VAT tertiles in both women and men. For isoprostanes, most of the relations with VAT appeared to be driven by those with the highest tertile of SAT.

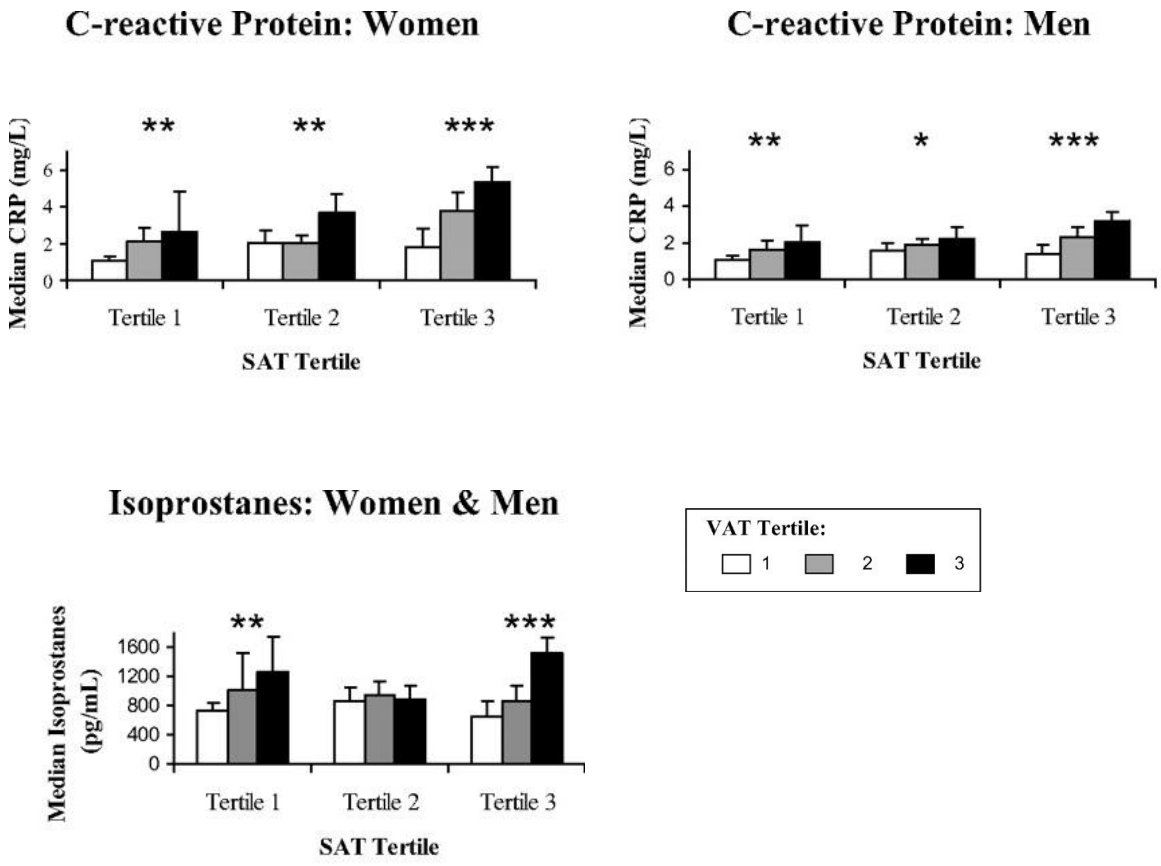

Figure 6. Sex-specific tertiles of VAT by sex-specific SAT tertiles for CRP; age-adjusted P value for linear trend is presented for women (upper left) and men (upper right). Lower left, Sex-specific tertiles of VAT by SAT tertiles for urinary isoprostanes for women and men combined; age- and sex-adjusted P value for linear trend is presented. Error bar represents upper 95% CI of the mean marker, and mean marker levels were back-transformed; the CRP data and the P values for trend are age-adjusted, and isoprostane data are age- and sex-adjusted.
*P-value for linear trend across VAT tertiles *<0.05, **<0.01, ***<0.001*

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In a secondary analysis, we also examined the significance of BMI and WL in multivariable-adjusted models with SAT or VAT in relation to the biomarkers (reflecting the models presented in Table 9). When we considered $P<0.01$ as indicating significance, for SAT, BMI was significant in the following models: CRP (women and men), fibrinogen, and IL-6, whereas WL was significant for osteoprotegerin and P-selectin (Table 11). For VAT, BMI was significant for CRP (women only), fibrinogen, and IL-6, whereas WL was not significant in any of the models.

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Table 12 - P-values for BMI and WL in multivariable-adjusted linear regression models of relation of SAT or VAT to biomarkers†

		BMI p-value	WL p-value
C-reactive protein			
Women	SAT	<0.0001	0.01
	VAT	<0.0001	0.16
Men	SAT	0.008	0.19
	VAT	0.02	0.38
CD40 Ligand	SAT	0.64	0.69
	VAT	0.79	0.95
Fibrinogen	SAT	0.004	0.83
	VAT	0.001	0.40
ICAM-1	SAT	0.08	0.22
	VAT	0.10	0.29
Interleukin-6	SAT	0.0004	0.83
	VAT	0.0004	0.71
Isoprostanes	SAT	0.30	0.02
	VAT	0.92	0.29
Lp-PLA2 activity	SAT	0.72	0.16
	VAT	0.40	0.48
Lp-PLA2 mass	SAT	0.76	0.99
	VAT	0.61	0.74
MCP-1	SAT	0.55	0.02
	VAT	0.25	0.12
Myeloperoxidase	SAT	0.76	0.67
	VAT	0.46	0.26
Osteoprotegerin	SAT	0.33	0.005
	VAT	0.16	0.02
P-selectin	SAT	0.50	0.002
	VAT	0.26	0.02
Tumor Necrosis Factor-α	SAT	0.25	0.48
	VAT	0.35	0.70
TNFR-2	SAT	0.007	0.80
	VAT	0.003	0.51

†Adjusted for sex, age, smoking, aspirin, alcohol intake, menopausal-status and hormone replacement (women only), and physical activity index. SAT (subcutaneous adipose tissue), VAT (visceral adipose tissue), Lp-PLA2 (lipoprotein-associated phospholipase A2 mass and activity), MCP-1 (monocyte chemoattractant protein-1), TNFR-2 (tumor necrosis factor receptor -2)

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4.4 Pericoronary adipose tissue quantification

The baseline characteristics of the 51 patients as stratified by the 3 groups are described in Table 12. Overall, traditional risk factors were most prevalent in group 1. Notably, hyperlipidemia was more frequent in Group 1 patients (plaque, hs-CRP>2 mg/L) compared to patients in Group 2 (no plaque, hs-CRP>2 mg/L) or Group 3 (no plaque, hs-CRP<1 mg/L) (p=0.01). BMI varied across the three studied groups, with the greatest BMI measured in Group 2 patients (no plaque, hs-CRP>2) (p=0.05). Additionally, the proportion of patients with hypertension tended to be decreased across the groups (p=0.09).

Table 12 - Demographics of overall cohort and matched groups

	Overall (n = 51)	Group 1 Plaque, hs-CRP>2 (n = 17)	Group 2 No plaque, hs-CRP>2 (n = 17)	Group 3 No plaque, hs-CRP<1 (n = 17)	p
Age (years)	49.5 ± 5.1	49.9 ± 5.5	49.0 ± 5.2	49.8 ± 4.9	0.87
Male (%)	33 (64.7)	11 (64.7)	11 (64.7)	11 (64.7)	1.0
Caucasian (%)	44 (86.3)	15 (88.2)	14 (82.4)	15 (88.2)	1.0
BMI (kg/m^2)	28.5 ± 4.9	29.1 ± 4.2	30.3 ± 6.2	26.3 ± 3.2	0.05
Diabetes (%)	4 (7.8)	3 (17.7)	0 (0)	1 (5.9)	0.31
Hypertension (%)	14 (27.4)	8 (47.1)	4 (23.5)	2 (11.8)	0.09
Hyperlipidemia (%)	15 (29.4)	10 (58.2)	2 (11.8)	3 (17.7)	0.01
Smoking (%)	29 (56.9)	12 (70.6)	8 (47.1)	9 (52.9)	0.46
hs-CRP (mg/L) [IQR]	2.62 [0.63-3.69]	3.86 [3.16-8.75]	2.88 [2.41-3.65]	0.39 [0.32-0.63]	<0.0001
hs-CRP > 2 mg/L (%)	34 (66.7%)	17 (100%)	17 (100%)	0 (0%)	<0.0001
GFR ($ml/min/1.73m^2$)	86.3 ± 13.2	90.0 ± 15.5	84.2 ± 14.0	84.6 ± 9.3	0.36

BMI denotes body mass index; hs-CRP, high sensitivity C-reactive protein; IQR, interquartile range; and GFR, glomerular filtration rate.

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4.4.1 Reproducibility of PCAT measurements

The average PCAT volume surrounding the coronary arteries was 26.98±13.33 cm^3 (range: 5.45 – 60.54 cm^3). Table 13 shows the excellent reproducibility of PCAT volume measurement with intra-observer and inter-observer ICC >0.94 overall and >0.87 on a per-vessel basis, with the least reliable PCAT quantification for the LCx artery. Figure 7 depicts Bland-Altman analysis of the individual reads by Observer 1 and 2 for PCAT volume on a per patient basis with good concordance and slight systematic bias at greater PCAT volumes.

Table 13 - Intra- and inter- observer reproducibility of PCAT measurement

	Intra-observer			Inter-observer		
	Mean Actual Difference (cm^3)*	Percent Difference	ICC†	Mean Actual Difference (cm^3)*	Percent Difference	ICC†
Overall	0.84 ± 1.93	3.4%	0.99	0.86 ± 4.30	3.4%	0.95
LAD	0.01 ± 0.47	0.1%	0.99	0.19 ± 1.02	3.0%	0.97
LCx	0.07 ± 0.90	1.1%	0.97	0.50 ± 2.12	7.5%	0.87
RCA	0.78 ±1.53	6.6%	0.98	0.16±2.65	1.3%	0.94

LAD denotes left anterior descending artery; LCx, left circumflex artery; RCA, right coronary artery; ICC, interclass correlation coefficient.
*All p-values are non-significant
† All p-values <0.0001.

4.4.2 Patient based PCAT analysis

In a patient-based analysis (Table 14), PCAT volume differed significantly across the three groups (p<0.0001), and was greater in patients with coronary plaque (Group 1) than no plaque irrespective of hs-CRP levels (Group 2: high hs-CRP, p<0.0001) and (Group 3: low hs-CRP, p<0.0001). Moreover, in patients without plaque, no difference in PCAT volume was seen between patients with high and low hs-CRP levels (p=1.0). The difference in PCAT volumes remained significant across the three patient groups after adjustment for BMI, hypertension, and hyperlipidemia (p=0.0002).

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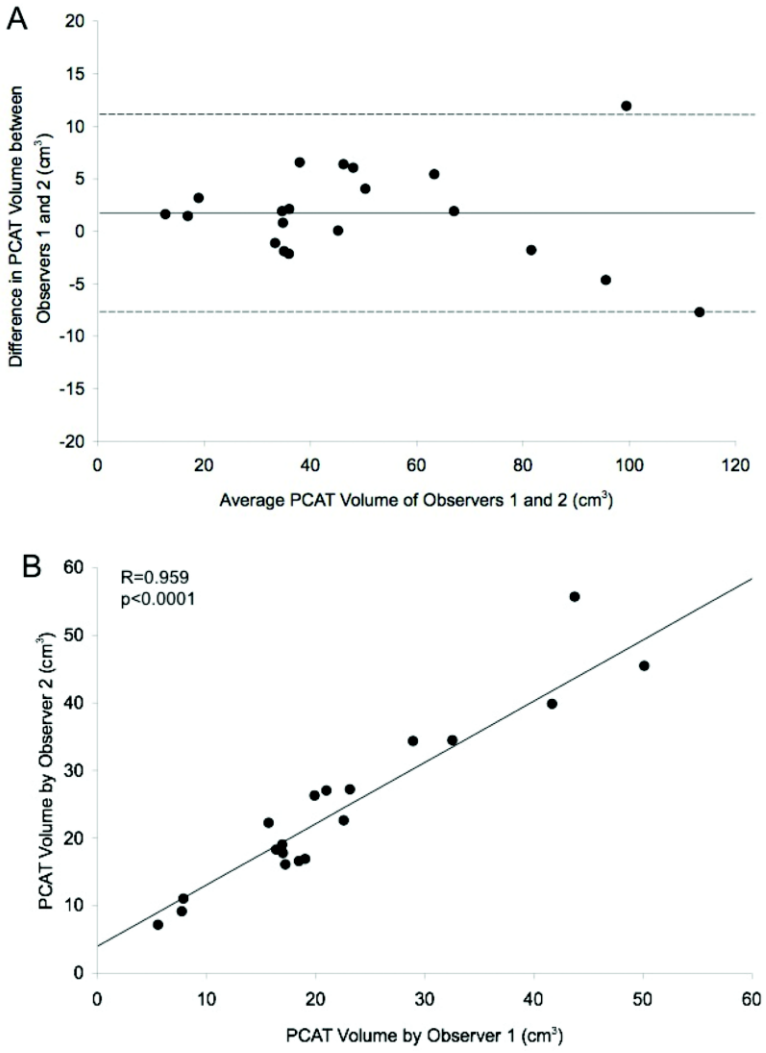

Figure 7 - Bland-Altman analysis (panel A) was used to assess the level of agreement between the two readers. A range of agreement was defined as mean bias ±2 SD (dashed lines) from the mean difference (solid line). Inter-observer correlation of volumetric measurements of pericoronary adipose tissue is shown in panel B.

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4.4.3 Vessel based PCAT analysis

A vessel-based analysis of PCAT was performed in 153 vessels including 51 left anterior descending, 51 left circumflex, and 51 right coronary arteries. The results were consistent with the patient-based analysis as a significant difference in PCAT volume was observed among the groups in all three vessel territories (Table 14). Mean PCAT volume was greatest surrounding the right coronary artery.

Table 14 - Patient and vessel based analysis and PCAT measures

PCAT Volume (cm^3)	Any Plaque hs-CRP >2	No Plaque, hs-CRP >2	No Plaque, hs-CRP <1	p value
Overall	38.6±11.8	21.3±10.0	21.0±10.1	<0.0001
LAD	10.4±5.8	6.0±2.5	5.8±3.0	0.0018
LCx	9.4±4.1	6.2±3.2	5.3±2.8	0.0044
RCA	17.7±5.3	12.5±7.6	10.0±4.8	0.0044

PCAT denotes pericoronary adipose tissue, hs-CRP, high sensitivity C-reactive protein; LAD, left anterior descending artery; LCx, left circumflex artery; RCA, right coronary artery.

4.4.4 Subsegment based PCAT analysis

To determine the relationship of local adipose tissue volume and coronary plaque distribution, a subsegment-based analysis was performed in 5 mm increments to assess PCAT quantity adjacent to 1224 vessel subsegments with and without plaque (Figure 8). No atherosclerotic plaque was detected in 1005 subsegments while 219 subsegments contained plaque. The median fat volume surrounding 5 mm vessel subsegments with plaque was higher compared to subsegments without plaque ($p<0.0001$).

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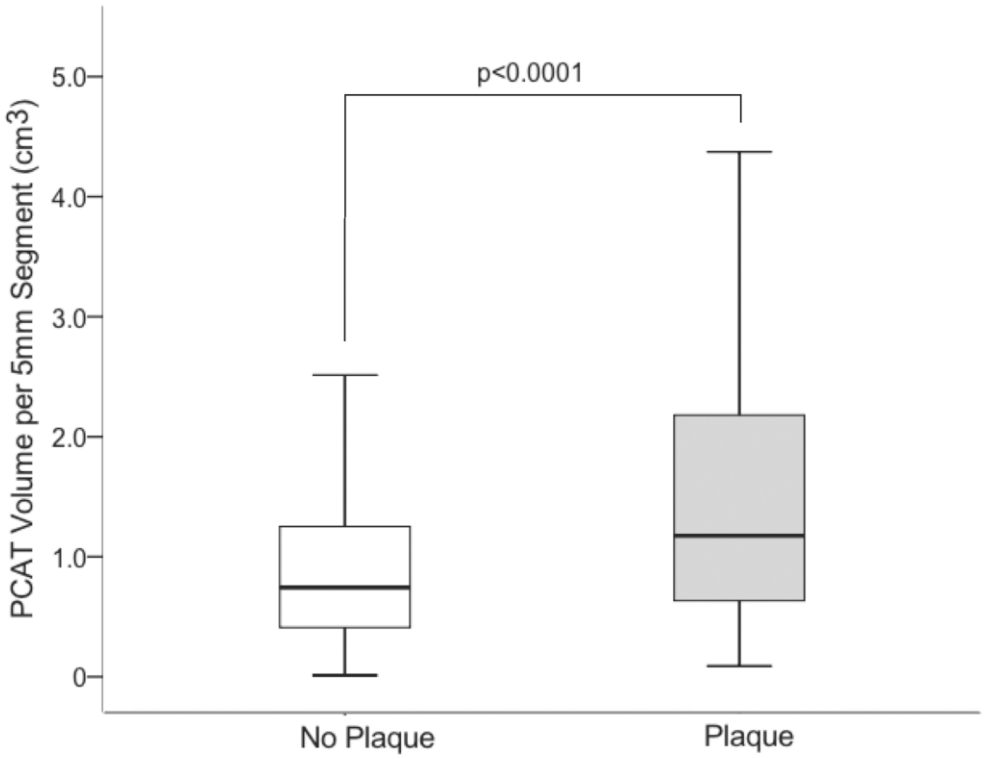

Figure 8 - The median segmental PCAT volume was higher adjacent to plaque-containing 5 mm coronary segments compared to segments free of atherosclerotic lesions

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5 Discussion

5.1.1 Abdominal adipose tissue, metabolic risk and inflammation

We demonstrated an excellent intra- and inter-observer reproducibility of MDCT-based volumetric quantification of subcutaneous and visceral abdominal adipose tissue in a community-based sample. We also showed a significant difference of the relative amounts of visceral and subcutaneous abdominal tissue between volumetric and planimetric measurements. Volumetric based adipose tissue compartment ratios depicted expected age and sex related differences in abdominal adipose tissue distribution. In addition, our data suggest that anthropometric measures of abdominal obesity such as BMI, SD and WL are well correlated to the absolute amount of abdominal adipose tissue but unrelated to the relative distribution of visceral and subcutaneous adipose tissue.

Our data demonstrate that both planimetric and volumetric methods for quantification of abdominal adipose tissue have excellent intra- and inter-observer reproducibility. Interestingly, more complex volumetric measurements had similar reproducibility to the much simpler planimetric measurements indicating that a method employing semi-automatic segmentation to identify the visceral and subcutaneous adipose tissue compartments was very accurate and effective. Volumetric measurements also proved to be very rapid with a total processing time of five minutes for all measurements. In addition, the quantification of anthropometric measurements such as SD and WL also appears to be highly reliable using CT, and comparison of CT measures with measurements obtained in the office setting are warranted.

One methodological subtlety is the allocation of intermuscular adipose tissue (IMAT) to either VAT or SAT. While some studies included IMAT in the SAT compartment, drawing the boundary between these two adipose tissue compartments along the internal abdominal wall (91-93), we traced the boundary close to the outer surface of the abdominal muscle wall, allocating IMAT within the VAT compartment similarly to previous studies (51,94-96). Our decision was based on previous observations that the characteristics of IMAT are closer to VAT than to SAT (97,98).

However, it is important to note that the amount of IMAT is very small compared to both SAT and VAT in the investigated region.

The comparison of absolute amounts of abdominal and visceral adipose tissue between planimetric-area based and volumetric methods in this study is limited to correlations since absolute volumetric measures from obtained over an axial length of 150 mm are naturally much larger than from a single 5 mm slice. In order to assess possible differences between these two methods for the distribution of subcutaneous and visceral adipose tissue we therefore used the relative distribution of subcutaneous and visceral adipose tissue expressed as a ratio between the two compartments. We found that volumetric measurements render significantly different adipose tissue compartment ratios than planimetric measurements (mean SAA/VAA ratio: 2.0 ± 1.2 vs. mean SAV/VAV ratio: 1.7 ± 0.9; $p<0.001$), although the planimetric and volumetric methods showed a reasonable intra class correlation (ICC=0.84). This difference was more evident in subjects with a high SAA/VAA ratio (≥ 2.5) where the SAV/VAV ratio was about 50% lower. In addition, the mean SAV/VAV ratio was significantly lower in older subjects, reflecting a redistribution of adipose tissue towards the visceral compartment with age. Above the age of 60 years, body weight on average tends to decrease, and this is mainly due to the loss of muscle mass with age (99). Furthermore, the body adipose tissue tends to be redistributed with advancing age toward more abdominal, particularly visceral adipose tissue (99,100). In our sample, men had significantly lower SAV/VAV ratios than women, reflecting the fact that men have higher relative amounts of visceral adipose tissue than women. Thus, volumetric measurements also appear to be useful to depict age- and gender specific abdominal adipose tissue distribution.

We hypothesize that these differences between the planimetric and volumetric method are a result of the inhomogeneous distribution of visceral and subcutaneous adipose tissue throughout the axial length of the abdomen (101,102).

Magnetic resonance imaging (MRI) also permits the quantification of visceral and subcutaneous adipose tissue with most accurate results derived from whole body analysis using contiguous slices (78,94,103,104). While both CT and MRI report good intra- and inter-reader reproducibility of abdominal adipose tissue quantification (94,98,101,103,104); MRI overestimated subcutaneous and visceral adipose tissue

compartments when compared to findings with CT (105,106). Superior accuracy of CT can be attributed to: 1) absolute CT values of pixels corresponding directly to the tissue property, whereas in MRI there is no direct association between tissue property and pixel value; 2) MRI based signal intensities may be inhomogeneous in particular at a larger FoV; and 3) better spatial resolution of CT. In addition, feasibility of MRI is limited due to long acquisition time and high expenses and thus MRI studies are usually performed on relatively small number of patients.

In a larger study sample, volumetric CT measures of both SAT and VAT were correlated with multiple metabolic risk factors. Risk factor correlations with VAT were consistently significantly stronger than those for SAT. VAT, not SAT, provided information above and beyond simple clinical anthropometrics, including BMI and WL, and consistently provided differences in risk factor stratification among individuals who were overweight and obese. VAT was more strongly associated with metabolic risk factors in women than in men.

VAT has traditionally been considered the more pathogenic adipose tissue compartment when compared to SAT, but data confirming these relations using high-resolution volumetric imaging assessments in large, community-based samples of women and men have been lacking. The mechanism of increased metabolic risk is hypothesized to be related to the metabolically active adipose tissue found in the visceral region (19,21-26,107), in addition to the drainage of these substances directly into the portal circulation (108). Multiple small studies have demonstrated that the visceral fat compartment is metabolically active, secreting such vasoactive substances as inflammatory markers (109), adipocytokines (23,110,111), markers of hemostasis and fibrinolysis (including plasminogen activator inhibitor-1) (112,113), and growth factors (including vascular endothelial growth factor) (114), which may contribute to its role in cardiometabolic risk factor manifestation (115,116).

Our results are consistent with these prior findings in small studies and extend these findings to a well-characterized, community-based sample of men and women in which we show that all cardiometabolic risk factors examined were more strongly associated with VAT than SAT. We also extend the current literature to note statistically significant, albeit weaker, correlations with SAT. Although SAT and VAT are highly

correlated with each other, we used the R2 (for continuous variables) and c statistic (for dichotomous variables) to determine the total variance explained by SAT and VAT. We also performed a formal test of the difference in the ß-coefficients for SAT compared with VAT in relation to the outcome variables, and in nearly every situation, the ß-coefficient from the regression model was stronger for VAT than for SAT. Of note, SAT volume is greater than VAT volume in both women and men. Therefore, although the effect sizes between VAT and risk factors may be higher, it is possible that SAT volume actually contributes to more absolute risk.

Of interest are recent findings from the Dallas Heart Study, which examined metabolic risk factors relations in 1934 black and white women and men with SAT and VAT as assessed by MRI (117). An important difference between our study and the Dallas Heart Study is the inclusion of percent body fat in regression models. Given that we do not have a measure of percent body fat, our findings are not directly comparable. Nonetheless, the results of Vega et al. (117) also show considerably higher R^2 for models that include VAT than for SAT, particularly among white participants, and increased R2 for models that include VAT above and beyond percent body fat and WL.

Our results show that both SAT and VAT are associated positively with prevalence of hypertension, but only VAT provides significant information above and beyond BMI and WL. Other studies have demonstrated relations between VAT and hypertension (28,32-34). Among Japanese Americans and whites, VAT but not SAT was associated with hypertension, even after adjustment for BMI and WL (28,32), whereas among blacks, both SAT and VAT were associated with hypertension in men and women (34), underscoring the relative importance of fat depots among different ethnic groups (55). We also found that both SAT and VAT were associated with triglycerides and HDL cholesterol in women and men. Our results build on those of others, which confirm the known association between VAT and lipids (35,37-39). However, we extend these findings to include strong and significant relations of SAT with HDL cholesterol and triglycerides. The primary difference with prior studies may be our large sample size in a community-based cohort compared with the few other studies that were adequately powered to compare the difference between SAT and VAT (35,39).

Similarly, for impaired fasting glucose and diabetes, multiple prior studies have

demonstrated relations between VAT and prediabetic hyperglycemia and diabetes (21,23,28,29), but few have yielded significant relations for SAT. However, SAT has been shown in multiple studies to be more strongly associated with insulin resistance than VAT (118). Some studies of insulin resistance have demonstrated stronger correlations with SAT than with VAT (30), especially in women. In the Insulin Resistance Atherosclerosis Study (IRAS), both SAT and VAT were important correlates of insulin resistance (31). One small study of 47 women and men demonstrated equivalent correlations of deep SAT and VAT with respect to cardiometabolic risk factors (119).

Although our results show that VAT is more highly correlated with MetS than SAT, SAT was an important correlate of the MetS. These findings are in contrast to prior studies, which have reported that SAT is only weakly associated with MetS. For example, in the Health, Aging, and Body Composition (Health ABC) Study, SAT was associated only with MetS in normal-weight and overweight men (93); however, unlike our study, the Health ABC Study focused primarily on older individuals (50). Therefore, SAT may be an important adipose tissue compartment that should not be overlooked. Only 1 very small intervention study has been conducted to date to examine the relation of SAT reduction with metabolic variables: In a small study of 15 women who underwent large-volume liposuction, despite the loss of nearly 10 kg subcutaneous fat, improvements in cardiometabolic risk factors were not observed (120). However, the small sample size, associated low power, and inclusion of morbidly obese study participants make it difficult to rule out a beneficial effect.

The strong correlation between SAT and cardiometabolic risk factors may be driven by the results from some (30,31,118), but not all (54,121) studies that have shown that insulin sensitivity is related to SAT and VAT. In addition to insulin resistance, relations between specific fat depots and adipocytokines may be responsible for mediating the relations with risk factors. In particular, leptin has been shown to be equally, if not more, correlated to SAT compared with VAT (121). Leptin also has been implicated in vascular dysfunction (122-125), which suggests another potential mechanism whereby SAT may be associated with cardiometabolic risk factors.

Despite the statistically significant results observed with both SAT and VAT, only VAT provided information above and beyond BMI and WL, suggesting that VAT may be

a unique pathogenic fat depot. Similar findings have been noted among Japanese Americans, for whom VAT but not SAT was associated with hypertension, even after adjustment for BMI and WL (28,32). Unfortunately, we were unable to analyze SAT in the same models as BMI and WL because of the high collinearity between the variables. In fact, the R2 of SAT versus BMI/WL is much higher for SAT than for VAT (0.76 versus 0.54 for men, 0.81 versus 0.64 for women).

In our study, we found evidence for sex interactions in that increasing volumes of SAT and of VAT were consistently and more strongly associated with more adverse risk factors levels in women than in men. To the best of our knowledge, our findings are the most comprehensive examination of sex differences reported to date. In the Health ABC Study, a significant sex interaction was observed between VAT and diabetes (23). Among women and men from the Quebec Family Study and the Health, Risk factors, Exercise Training, and Genetics (HERITAGE) Family Study, only in women were higher amounts of VAT associated with adverse CVD risk factor profiles (126). The cause of these sex differences is uncertain but may be related to the higher amount of hepatic free fatty acid delivery derived from lipolysis from VAT that has been observed in women than in men (107).

The relation of MetS and its components with increasing VAT quartiles, particularly in overweight and obese individuals, suggests that VAT in particular may confer increasing risk within clinically defined categories of body weight. Two thirds of our study sample were either overweight or obese, statistics that mirror the North American data (6). Our data suggest that further observational and possibly interventional studies are warranted to test the impact of weight reduction and, in particular, the reduction of VAT on metabolic risk factors and overall CVD risk. Additionally, our work demonstrates strong and significant results for SAT and VAT in relation to cardiometabolic risk factors, suggesting that SAT should not be overlooked in regional adipose tissue research. Nonetheless, we note that the proportion of overall variation of VAT and of SAT with metabolic risk factors is moderate at best. This finding, which has been observed previously (117), suggests that other factors not accounted for in this study may be responsible for the variation in metabolic risk factors. In fact, many of these traits have a substantial heritable component, with reported heritabilities for glucose being 34%

to 51% (127); for systolic blood pressure, 53% (128); and for total cholesterol, 40% (129). Therefore, the potential role of gene–adiposity interactions should be considered in future research.

The analysis of the participants from the Framingham Offspring Multi-Detector CT Study revealed that CT-based measures of abdominal adiposity were significantly associated with several systemic biomarkers of inflammation and oxidative stress in women and men. Whereas most markers were similarly associated with SAT and VAT, isoprostanes and MCP-1 were more strongly associated with VAT than with SAT. When both SAT and VAT were considered in the same model, CRP, fibrinogen, and IL-6 remained correlated with both adipose tissue depots, whereas other markers were associated only with SAT or VAT. In addition, the association between VAT and CRP, IL-6, isoprostanes, and MCP-1 concentrations remained significant after we accounted for clinical measures of overall and central adiposity, which suggests that VAT may be a critical correlate of inflammation that is not completely accounted for by BMI and WL. We also observed that abdominal adiposity was more strongly associated with CRP in women than in men. Finally, we did not find an association between either SAT or VAT with circulating concentrations of CD40 ligand, Lp-PLA2 activity or mass, osteoprotegerin, or TNF-α.

The present finding that CRP was positively and similarly correlated with both SAT and VAT agrees with previous findings in small cross-sectional studies (109,130,131). Lemieux et al. (109) reported positive correlations between SAT and VAT with CRP concentrations (r=0.28 for SAT and r=0.33 for VAT) in 159 healthy middle-aged white men. Similar strong relations were reported in a sample of 112 white postmenopausal women (132). We observed a significant sex interaction for CRP, because both SAT and VAT were more strongly associated with CRP in women than in men. Whereas two recent cross-sectional studies suggested stronger associations between clinical measures of adiposity and markers of inflammation in white women than in men (133,134), we extended these findings of sex differences to relations of CT-based measurements of abdominal fat distribution and inflammation. Additionally, we found that VAT remained associated with CRP after adjustment for BMI and WL in both men and women. Therefore, whereas both SAT and VAT are correlates of CRP

concentrations, the present data suggest that VAT may be a critical correlate that is not completely accounted for by routine clinical measurements.

It has been proposed that a mechanistic link between adiposity and CRP may be explained by IL-6 and TNF-α (135-137). Both cytokines are produced in adipose tissue (138,139), are upregulated in obese states (133,140), and induce hepatic production of CRP (120,141). Some cross-sectional studies demonstrate stronger associations of IL-6 and TNF-α with abdominal girth (133,134) or visceral fat area (142) than with BMI, yet correlations between circulating concentrations of IL-6 or TNF-α and radiographic measures of fat distribution have not been well studied. We found that SAT and VAT were similarly related to IL-6 and that this relation persisted for VAT alone after adjustment for BMI and WL. TNF-α, however, was not associated with either SAT or VAT. Although it has been shown that adipose tissue from obese individuals expresses 2.5-fold more TNF-α mRNA than is expressed in lean control subjects (143), tissue RNA expression is not always reflected in circulating concentrations of the protein (144). Whereas ≈30% of circulating IL-6 originates in adipose tissue, systemic release of TNF-α is much more variable and is believed to function primarily in a paracrine fashion (145). However, we did find strong correlations with circulating concentrations of the soluble receptor TNF receptor-2 and both SAT and VAT in the present study, consistent with relations between TNF receptor-2 and obesity (146).

In addition to adipocytes, inflammatory cells such as monocytes and macrophages are components of adipose tissue and accumulate in obese states (147). Macrophages secrete MCP-1, and in human adipose tissue, MCP-1 mRNA concentrations are correlated with measures of adiposity, with higher expression in VAT than in SAT (148). Furthermore, circulating concentrations of MCP-1 are elevated in obesity and fall with exercise and loss of visceral fat (149). Adding to the current literature, we found that MCP-1 was more strongly associated with VAT than with SAT and that VAT may contribute to MCP-1 variability beyond the contributions of BMI and WL.

Oxidative stress, as reflected by isoprostanes (a metabolite of lipid peroxidation that serves as a time-integrated marker of oxidative stress) (150) and myeloperoxidase (an oxidative enzyme produced by macrophages), has been positively associated with obesity in a few studies that assessed central obesity via waist-hip ratio or estimated visceral fat

with bioelectric impedance (85,151,152). In the present study, myeloperoxidase was correlated with both SAT and VAT. A prior small study has shown a decrease in both myeloperoxidase and isoprostanes with a diet and exercise intervention in obese men (152), but we believe the present finding of myeloperoxidase in relation to SAT and VAT is novel. Furthermore, the results of the present study are among the first to show a direct correlation of urinary isoprostane concentrations and SAT or VAT, with stronger correlations observed for VAT than for SAT. We also show that isoprostanes remained associated with VAT after adjustment for BMI and WL. The present data support the hypothesis that visceral adiposity is a unique correlate of oxidative stress.

Although a few small studies have reported high concentrations of soluble ICAM-1 (153) and P-selectin (154) in the plasma of obese individuals, the relation of these adhesion molecules to fat distribution has not been well studied. ICAM-1 has been demonstrated to be associated with BMI and waist-hip ratio (153). We found that SAT and VAT were similarly associated with circulating concentrations of ICAM-1 and with P-selectin. Neither of these relations remained significant after adjustment for BMI and WL. Therefore, it does not appear that the relation between either ICAM-1 or P-selectin and visceral adiposity is independent of clinically assessed anthropometry.

In the present study, we did not find an association between either SAT or VAT and circulating concentrations of CD40 ligand, Lp-PLA2 activity or mass, osteoprotegerin, or TNF-α . Although we used a precise measure of abdominal fat masses, our measures of inflammatory markers are limited to those present in the peripheral circulation. It is possible that some markers may be metabolized by the liver and may show a different association if they are measured in the portal circulation (137). Fontana et al. (137) recently demonstrated that IL-6 concentrations are 50% higher in portal vein samples than in those from the radial artery in obese women. However, it is notable that we found clear associations between SAT and VAT and circulating concentrations of many markers but no association between others. The present investigation of SAT was limited to SAT in the abdominal area, and thus, we cannot comment on associations between inflammatory markers and SAT in other regions.

The present findings underscore the positive association of both SAT and VAT with circulating markers of inflammation. Prior literature has emphasized the important

relations of visceral adiposity with inflammation and related cardiometabolic risk, whereas we show that both SAT and VAT appear to be associated with chronic inflammation. Therefore, SAT may have multiple metabolic and endocrinologic properties that have previously been ascribed only to VAT; this warrants investigation in further studies. To lend a clinical perspective to the present findings, we use a conversion factor of 0.9255 (155) to convert adipose tissue volume to mass. In women, SAT mass of 1.3 kg and VAT mass of 0.8 kg represents 1 SD; this small increase in adipose tissue mass corresponds to an increase in CRP concentration of 1.7 mg/L for each SD of SAT and 1.8 mg/L for VAT. In men, 1.0 kg of SAT and VAT corresponds to 1 SD, and the corresponding CRP concentrations are 0.6 mg/L higher for each 1-kg increase of SAT and 0.7 mg/L for each additional kilogram of VAT. Thus, a small increase in abdominal adipose tissue relative to overall body weight is associated with significantly elevated CRP concentrations, and this relation is most marked in women.

5.1.2 Pericoronary adipose tissue quantification

We described a novel three-dimensional (3D) quantification method to assess PCAT volume. This threshold-based method is similar to techniques used for coronary artery plaque quantification and characterization(156,157). Our technique allows for adipose tissue assessment along the course of the coronary artery through the generation of a three-dimensional color-coded map of voxels containing adipose tissue. The volumetric quantification of the adipose tissue compartment is achieved by summing voxels within the predefined HU range. The strength of our method is that it allows true volumetric assessment, and a detailed analysis of short PCAT subsegments and their relation to underlying coronary atherosclerotic plaque. Furthermore, it has an excellent intra- and inter-observer reproducibility, which is in line with several other studies utilizing volumetric measurements for adipose tissue quantification in different body regions (58,65,86,158). The most commonly used techniques for PCAT quantification in cardiac CT datasets are based on two-dimensional (2D) measurements of thickness and area, both of which are measured using a limited number of axial slices (65,67-69). The reproducibilities of 2D metrics have been modest, with coefficient of variation between

12.0% - 23.4% and inter-observer ICC of 0.76 (95% CI 0.50 – 0.88) (65,67,68). Moreover, these 2D quantification techniques may not account for the inhomogeneous distribution of PCAT along the atrioventricular and interventricular grooves due to the limitations posed by the single CT cross-sectional assessment. These limitations could explain the conflicting data on the association of PCAT thickness and coronary atherosclerosis (67,68). A recently published PCAT quantification method by Mahabadi et al relies on tracing and summing PCAT areas in the original axial slices (158). Whereas our method assesses PCAT by tracing regions containing adipose tissue in planes perpendicular to the vessel centerline, thus allowing a true volumetric assessment. Furthermore, Mahabadi et al excluded PCAT surrounding the RCA due to limitations of the method they used (158). Notably we found that the quantification of the RCA territory actually yielded the highest PCAT volumes. Our results indicate that elevated PCAT volume is related to plaque presence on a patient and vessel based evaluation in the LAD, LCx, and RCA vessel territories. These observations contribute to the increasing body of evidence that there is an association between PCAT and coronary artery disease (65,67,68,158). We believe it is important to quantify PCAT volumes at the subsegmental level to assess the relationship between plaque and directly adjacent adipose tissue.

In addition, we stratified the cohort according to hs-CRP levels and presence of plaque in order to assess the systemic effects of inflammation on the relationship between PCAT volume and coronary artery disease. We found that patients without plaque had similar PCAT volumes despite differences in hs-CRP levels. These findings are in line with previous studies suggesting local processes in addition to systemic effects in the development of coronary atherosclerosis (61,64,67). Mechanistically, adipocytokines secreted by the pericoronary adipose tissue may diffuse into the vasculature and lead to the development of atherosclerotic changes in the adjacent vessel wall via local inflammatory effects, thus plasma inflammatory markers may not adequately reflect local tissue inflammation and atherogenesis (61). Further larger studies are needed to examine the effect of local adipocytokines on atherosclerotic plaque development.

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5.1.3 Strengths and limitations

Strengths of our study include the use of a community-based sample with participants not enriched for adiposity-related traits. Routine assessment of clinical characteristics was performed, which allowed for adjustment for several potential confounders. We used a highly reproducible volumetric method of SAT and VAT assessment, which accounts for heterogeneity of fat distribution throughout the abdomen.

We were able to assess the role of SAT and VAT above and beyond clinical anthropometry. Our study participants were primarily middle-aged, allowing assessment of relations between fat compartments and metabolic risk factors. Furthermore we were able to investigate a broad panel of circulating markers of systemic inflammation that represent various steps along the inflammatory pathway. Lastly, we have a large sample with adequate power to detect potentially smaller but significant relations with abdominal adipose tissue compartments.

Limitations include the cross-sectional design; because the associations are not prospective, causality cannot be inferred. Because the Framingham Heart Study sample is primarily a white sample, generalizability to other ethnic groups is uncertain. For example, Japanese Americans and Southeast Asians have groups with more visceral fat than expected for a given overall BMI (55), whereas blacks have less visceral fat than do whites for a given BMI (159). Although we accounted for sibling–sibling correlations, current analytical methods did not allow us to account for all familial relations.

Additionally, risk factors and anthropometric data were not obtained contemporaneously with the CT data; this could potentially have caused us to underestimate the magnitude of the association between the markers and the adipose tissue compartments; however, this should not affect the relative association between SAT versus VAT and the marker concentrations, which was the primary focus of the present study. Furthermore, we measured systemic and not portal concentrations of biomarkers; therefore, biomarkers that may have been upregulated locally or substantially metabolized by the liver may not have been detected accurately in the present study.

Because we did not subdivide subcutaneous fat into superficial and deep compartments, we cannot comment on the relative importance of these compartments.

Furthermore, we measured only abdominal, not truncal, SAT. Truncal SAT has been shown to be more correlated to insulin resistance than is abdominal SAT in men (118).
Furthermore, there are some limitations regarding the described PCAT quantification methods. Since our case-control matching was performed within the ROMICAT cohort, we did not include analysis on a fourth group (patients with coronary plaque but low hs-CRP levels) due to inadequate number of patients for age- and gender-matching. While the adjusting for waist circumference or other proxy of visceral adiposity would be of interest, this data was not obtained in the ROMICAT study and thus unavailable. The results of this small feasibility study require validation in a larger dataset and may allow for sufficient number to examine this interesting fourth group. Our findings may not be applicable to distal vessels beyond 40 mm or left-dominant coronary systems. In addition, the LM was counted towards the LAD, thus the proportion of the investigated LAD might be relatively smaller than for RCA and CX. Since the measurement software used to assess PCAT was dedicated to coronary plaque evaluation with a limited range of HU settings, the lower limit of the HU value for fat quantification was defined by the lowest selectable HU value (-149 HU) in the software application. Furthermore, due to the partial overlap of the quantified adipose tissue volumes around at left main stem bifurcation, double sampling of the fat containing voxels occurs in some of the proximal LAD and CX subsegments. These limitations could be addressed by the future development of dedicated software for PCAT volume assessment.

6 Conclusions

We demonstrated an excellent intra- and inter-observer reproducibility of MDCT based volumetric quantification of subcutaneous and visceral abdominal adipose tissue. We also demonstrated significant differences of the relative amounts of visceral and subcutaneous abdominal tissue between volumetric and planimetric measurements. Although both SAT and VAT are correlated with metabolic risk factors, VAT remains more strongly associated with an adverse metabolic risk profile even after accounting for standard anthropometric indexes. Our findings are consistent with the hypothesized role of visceral

fat as a unique, pathogenic fat depot. Measurement of VAT may provide a more complete understanding of metabolic risk associated with variation in fat distribution. Furthermore, we found an association between both SAT and VAT with inflammation and oxidative stress. The data suggest that the contribution of visceral fat to inflammation may not be completely accounted for by clinical measures of obesity (body mass index and waist circumference).

Measurement of VAT and SAT may provide a more complete understanding of metabolic and cardiovascular risk, and further studies are warranted to prospectively assess the impact of the lowering of VAT and SAT on the incidence of MetS and CVD. Prior literature has emphasized the important relations of visceral adiposity with inflammation and related cardiometabolic risk, whereas we show that both SAT and VAT appear to be associated with chronic inflammation. Therefore, SAT may have multiple metabolic and endocrine properties that have previously been ascribed only to VAT.

Furthermore, we demonstrated the feasibility and excellent reproducibility of a novel threshold-based method for PCAT volume assessment. The local amount of PCAT volume was increased in the presence of plaque at the patient, vessel, and subsegmental level. In patients without plaque, PCAT volume did not differ based on hs-CRP level, highlighting the importance of local PCAT depots in addition to systemic inflammatory processes. Further studies are needed to validate our findings and elucidate the role of local adipocytokines on atherosclerosis.

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7 Summary

Excess abdominal adiposity is associated with increased morbidity and mortality. The incremental utility of measuring both visceral and subcutaneous adipose tissue in association with metabolic risk factors, markers of inflammation and oxidative stress has not yet been described in a population-based setting. Furthermore, the feasibility and reproducibility of the volumetric abdominal and pericoronary adipose tissue quantification techiques have not been reported.

We demonstrated that the volumetric quantification of subcutaneous and visceral abdominal adipose tissue with multidecetor-row CT has an excellent intra- and inter-observer reproducibility. The three-dimensional quantification method of the abdominal adipose tissue yielded a significantly different visceral and subcutaneous fat distribution compared to the two dimensional planimetric approach. The amount of visceral fat showed a significant correlation with the metabolic risk factors, even after adjustment for the body mass index and waist circumference. This finding is in line with the results of previously published data suggesting that visceral fat is a unique, pathogenic fat depot. Furthermore, we found an association between both subcutanous and visceral fat with inflammation and oxidative stress. After adjusting for the clinical measures of obesity visceral fat remained associated with several markers inflammation and oxidative stress, which suggests that systemic inflammation observed in obese individuals may not be completely accounted for by body mass index and waist circumference. Thus, the quantification of abdominal fat compartments may provide a more complete understanding of metabolic risk and systemic inflammation associated with variation in fat distribution.

Furthermore, we demonstrated the feasibility and excellent reproducibility of a novel threshold-based method for pericoronary adipose tissue assessment. The local amount of pericoronary fat showed an association with the local coronary atherosclerosis suggesting a relationship between pericoronary fat and coronary atherogenesis.

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8 Összefoglalás

A hasi elhízás fokozott morbiditással és mortalitással társul. A visceralis és a subcután zsírszöveti kompartmentek mérésének és a metabolikus rizikófaktorokkal, gyulladásos és oxidatív stressz-markerekkel való összefüggésének epidemiológiai vizsgálata ez idáig nem történt meg. Továbbá az abdominális és pericoronáriás zsírszövet volumetriás mérésének kivitelezhetősége és a mérés reprodukálhatóságának vizsgálata is tisztázásra szorul.

Munkámban bizonyítottam, hogy a subcutan és visceralis zsírszövet volumetriás meghatározása MDCT-vel kiváló intra- és interobserver reprodukálhatósággal rendelkezik. A visceralis és subcutan zsírszövet szignifikánsan különböző eloszlást mutatott a háromdimenziós mérési módszerrel a planimetriás meghatározáshoz képest. A visceralis zsírszövet mennyisége szignifikáns korrelációt mutatott a metabolikus rizikófaktorokkal BMI-re és csípőkörfogatra való korrigációt követően is. Ezen megfigyelés összhangban van a korábbiakban leírt megfigyelésekkel, amelyek szerint a viscerális zsírszövet patogén zsírkompartmentnek felel meg. Összefüggést találtunk mind a subcutan, mind a visceralis zsírszöveti mennyiségek és az inflammációs és oxidatív stressz-markerek szintje között. Az obezitás antropometriai paramétereire való korrekciót követően kizárólag a viscerális zsírszöveti kompartment mutatott összefüggést számos gyulladásos és oxidatív stressz-markerrel. Ezen megfigyelés arra utal, hogy az obezitásban megfigyelhető szisztémás gyulladás nem jellemezhető kizárólag a BMI és a haskörfogat mérésével. A volumetriás mérési módszereink hozzájárulhatnak a metabolikus rizikó, a szisztémás gyulladás és a hasi zsíreloszlás összefüggésének pontosabb megértéséhez.

Munkám második felében a pericoronáriás zsírszövet egy újfajta, CT-denzitás alapú mérésének kivitelezhetőségét és kitűnő reprodukálhatóságát demonstráltam. A pericoronáriás zsírszövet mennyisége összefüggést mutatott a coronaria-atheroscerosissal lokálisan, amely a pericoronáriás zsírszövet és az atherogenesis kapcsolatára utalhat.

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10 Publications

10.1 Publications closely related to the present thesis

Original articles

1. **Maurovich-Horvat P**, Massaro JM, Fox CS, Moselewski F, O'Donnell CJ, Hoffmann U: Comparison of Anthropometric, Area and Volume based Assessment of Abdominal Subcutaneous and Visceral Adipose Tissue Volumes using Multi Detector Computed Tomography. Int J Obes 2007;31:500-6. **IF: 3.56**

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Appendix (Papers 1-3)