Development and Reproducibility of a Computed Tomography–Based Measurement for Upper Body Subcutaneous Neck Fat

Klara J. Rosenquist, MD; Kate E. Therkelsen, BA; Joseph M. Massaro, PhD; Udo Hoffmann, MD, MPH; Caroline S. Fox, MD, MPH

Background—Upper body subcutaneous neck fat (UBSF) is a unique fat depot anatomically separate from visceral abdominal fat that appears to be associated with cardiometabolic risk above and beyond generalized adiposity. We sought to develop a protocol to quantify UBSF using multidetector computed tomography measurements.

Methods and Results—Protocol development was performed in participants from the Framingham Heart Study who had participated in the multidetector computed tomography scanning substudy, consisting of chest scans. Volumetric assessment of UBSF was defined by 40 contiguous 0.625-mm slices superior to the body of the sternum. The reader manually traced the chest to identify total neck fat. Breast tissue external to the chest wall was excluded. Subcutaneous and visceral fat volumes were obtained using standard protocols. Age- and sex-adjusted Pearson correlation coefficients were used to assess the association among UBSF, traditional adiposity measures, and cardiometabolic risk factors. Inter- and intrareader reproducibility was assessed using intraclass correlation coefficients. Volumetric assessments were obtained in 92 participants because 8 scans were not readable (51% women; mean age: 59 years [women], 58 years [men]). The mean volume of UBSF was 310 cm³ for women and 345 cm³ for men. Intra- and interreader class correlation coefficients were 0.99 and 0.99, respectively. UBSF was correlated with waist circumference (r=0.90), neck circumference (r=0.75), body mass index (r=0.89), subcutaneous adipose tissue (r=0.87), and visceral adipose tissue (r=0.86).

Conclusions—UBSF can be quantified reproducibly using computed tomography in a community-dwelling sample from the Framingham Heart Study. (J Am Heart Assoc. 2014;3:e000979 doi: 10.1161/JAHA.114.000979)

Key Words: fat distribution • multidetector computed tomography • upper body subcutaneous neck fat

The prevalence of obesity in the United States has reached epidemic proportions and continues to rise.¹ Numerous harmful sequelae of obesity including increased cardiometabolic risk²–⁴ and overall mortality⁵ have been demonstrated. Variations in body fat distribution, independent of generalized adiposity, may be associated with differential metabolic risk. Visceral adipose tissue (VAT) is a fat depot that has been associated with an increased risk of metabolic syndrome, insulin resistance, and type 2 diabetes.²,³ However, studies have demonstrated only modest correlations between cardiometabolic risk factors and visceral adiposity, leading investigators to hypothesize that other fat depots may confer additional risk for the development of cardiovascular disease.²,⁶ Upper body subcutaneous neck fat (UBSF) is a unique fat depot located in a separate anatomic compartment and has been associated with insulin resistance independent of VAT.⁷,⁸ Consequently, this fat depot may be associated with differential cardiometabolic risk above and beyond generalized adiposity and visceral fat.

Traditional measures of obesity have included anthropometric measurements including body mass index (BMI) and waist circumference, both of which have been correlated with increased risk of obesity-related morbidity.²,⁹ Further research, including data from our group, has shown that neck circumference has been correlated with cardiome-
bolic risk factors even after adjusting for levels of visceral adiposity and BMI, suggesting the significance of UBSF in the pathogenesis of obesity. In addition, we have also shown that neck circumference is associated with carotid atherosclerotic disease above and beyond other measures of adiposity; however, neck circumference is only a proxy for UBSF. We aimed to develop a protocol to quantify UBSF using multidetector computed tomography (MDCT) in a community-dwelling sample from the Framingham Heart Study.

Methods

Study Participants

The original Framingham Heart Study cohort was first established in 1948 with the original cohort recruited from Framingham, Massachusetts. The Offspring and Third Generation cohorts were recruited subsequently and have been well described previously.

The MDCT substudy consists of 1333 participants from the Offspring cohort and 1431 participants from the Third Generation cohort who underwent computed tomography imaging as part of a comprehensive assessment of vascular calcification. Participants for our current analysis are drawn from the MDCT 2 substudy who underwent scanning from September 2008 to December 2011. Clinical characteristics were obtained at Offspring exam 8 and Third Generation exam 2. Inclusion criteria included an age of >35 years for men and >40 years for nonpregnant women. All participants weighed <450 lb because of MDCT scanner specifications.

A sample of 100 participants was selected from the study population for the purposes of the present analysis. This sample was randomly selected to include 50 women and 50 men evenly spread across the ages and divided between the Offspring and Third Generation cohorts. The institutional review boards of Boston University Medical Center and Massachusetts General Hospital approved the study protocol, and all participants provided written consent.

MDCT Scan Acquisition

Subjects underwent MDCT imaging of the thorax in a supine position using a General Electric Discovery VCT 64-slice PET/CT scanner (GE Healthcare). The entire chest from the lung base to the apices was imaged using a prospectively ECG-triggered computed tomography scanning protocol during a single inspirational breath hold to enable lung and coronary assessment of the apices. The gantry rotation time was 0.35 seconds, the tube voltage was 120 kVp, and the tube current was 300 mA (subject weight ≤220 lb) or 350 mA (subject weight >220 lb). A detector width of 0.625 mm was used. The 210° scan reconstruction algorithm was used, providing 210° of raw scan data (tube rotation) with an optimized reconstruction technique that provided images of ≈175-ms temporal resolution depending on the speed of gantry rotation (0.35 seconds).

Quantification of Upper Body Subcutaneous Neck Fat

MDCT slices were loaded onto a dedicated offline workstation (Aquarius 3D workstation; TeraRecon Inc). A predefined image display setting (−195 to −45 Hounsfield units; center: −120 Hounsfield units) was used to identify adipose tissue within these slices.

The anatomic region of interest was defined by 40 contiguous 0.625-mm slices superior to the body of the sternum and covering an area of 25 mm. The sternal landmark was determined by the first single slice to show the bowtie shape of the sternum filled completely with trabecular bone (Figure 1A). Forty contiguous slices above the sternum were selected, and every 10th slice starting with the first slice was manually reviewed. The reader manually excluded the adipose tissue within the mediastinum (Figure 1B) for the total neck fat. The reader then outlined the adipose tissue exterior to the chest wall (Figure 1C) to exclude breast tissue. The final region of interest was defined as UBSF and was volumetrically measured (cubic centimeters). The full protocol appears in Data S1.

Assessment of Covariates

BMI, waist circumference, neck circumference, systolic blood pressure, diastolic blood pressure, fasting plasma glucose, and fasting lipids were determined based on study visit evaluations. BMI was calculated by dividing the weight (kilograms) by the square of height (meters). Waist circumference was measured to the nearest 0.25 inch at the level of the umbilicus. Neck circumference was measured to the nearest 0.25 inch below the laryngeal prominence and perpendicular to the long axis of the neck. Plasma glucose, insulin, and lipids were measured on fasting morning samples.

Diabetes was defined as a fasting plasma glucose ≥126 mg/dL or treatment with insulin or a hypoglycemic agent. Participants were considered to be current smokers if they smoked ≥1 cigarettes per day for the last year.

Among the representative preselected sample of 100 participants used for this analysis, 2 scans did not have adequate slices through the anatomic region of interest, 3 scans did not have the required 40 scans superior to the
sternal landmark, and 3 had unusual anatomy disrupting the landmarks, resulting in a readable sample size of 92. This sample size of 92 was used to determine reproducibility. One participant did not attend the clinical examination, and no baseline characteristics were available; therefore, the sample characteristics are based on a sample size of 91.

Abdominal subcutaneous adipose tissue (SAT) and VAT volumes were previously determined using a protocol for abdominal adipose tissue quantification that was described previously.13

Statistical Analysis

The protocol was completed independently by 2 readers (K.J.R., K.E.T.) for interobserver variation and then repeated by the first reader (K.J.R.) for intraobserver variation. Inter- and intrar reader reproducibility was evaluated using intraclass correlation coefficients. Pearson correlation coefficients were calculated to determine the association between UBSF and cardiometabolic risk factors. Statistical analyses were performed using SAS version 9.2 (SAS Institute Inc).

Results

Sample Characteristics

Baseline characteristics for the sample are presented in Table 1. The mean age was 58.8 years for women and 57.9 years for men. Women composed 51% of the sample. The mean BMI was 27.1 kg/m² for women and 27.8 kg/m² for men.

Distribution of Upper Body Subcutaneous Neck Fat

The mean volume of total neck fat was 438 cm³ for women and 429 cm³ for men. The mean volume of breast tissue fat was 128 cm³ for women and 83 cm³ for men. The anatomic area of interest, UBSF, was defined by the total neck fat minus the breast fat (Figure 1). The mean volume of UBSF was 310 cm³ for women and 345 cm³ for men.

Intra- and Interreader Reproducibility

Intra- and interreader measurements are plotted in Figure 2. The intrar reader intraclass correlation coefficient was 0.99, and the interreader intraclass correlation coefficient was 0.99.

Correlation of Upper Body Subcutaneous Neck Fat With Cardiometabolic Risk

Age- and sex-adjusted Pearson correlation coefficients for UBSF and cardiometabolic risk factors are presented in Table 2. UBSF was strongly correlated with waist circumference ($r=0.90$), BMI ($r=0.89$), SAT ($r=0.87$), and VAT ($r=0.86$). UBSF also correlated with neck circumference ($r=0.75$).

Discussion

The findings of this study are 2-fold. First, the quantification of UBSF using computed tomography is feasible and reproducible
Second, UBSF is correlated with cardiometabolic risk including strong correlations with other measures of adiposity in our feasibility study. Traditional measures of obesity include anthropometric measurements such as BMI and waist circumference, both of which have been correlated with increased cardiometabolic risk and increased risk of obesity-related morbidity.²,⁹

<table>
<thead>
<tr>
<th>Continuous characteristics</th>
<th>Women (n=45)*</th>
<th>Men (n=46)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>58.6 (10.0)</td>
<td>57.6 (11.1)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.1 (5.0)</td>
<td>27.8 (4.7)</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>96.7 (14.4)</td>
<td>100.2 (11.8)</td>
</tr>
<tr>
<td>Neck circumference, cm³</td>
<td>33.6 (2.2)</td>
<td>41.3 (3.1)</td>
</tr>
<tr>
<td>VAT, cm³</td>
<td>1589 (943)</td>
<td>2784 (1328)</td>
</tr>
<tr>
<td>SAT, cm³</td>
<td>3672 (1605)</td>
<td>2779 (1266)</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>97 (10.1)</td>
<td>105 (30.4)</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>119 (14.1)</td>
<td>125 (17.2)</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>63 (19.6)</td>
<td>52 (16.3)</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>120 (165.3)</td>
<td>122 (95.3)</td>
</tr>
<tr>
<td>Total neck fat</td>
<td>438 (108.7)</td>
<td>429 (86.8)</td>
</tr>
<tr>
<td>Breast fat</td>
<td>128 (45.6)</td>
<td>83 (25.4)</td>
</tr>
<tr>
<td>Upper body subcutaneous neck fat ³</td>
<td>310 (71.0)</td>
<td>345 (65.6)</td>
</tr>
</tbody>
</table>

Categorical characteristics

| Current smoker, % | 6.5 | 13.3 |
| Diabetes, %       | 0   | 6.7  |

Data presented as mean (SD) for continuous characteristics and mean (SD) or median (quartile 1, quartile 3) for categorical characteristics. BMI, indicates body mass index; HDL, high-density lipoprotein; SAT, subcutaneous adipose tissue; SBP, systolic blood pressure; VAT, visceral adipose tissue.

*Based on a sample size of 91 because 1 participant did not attend the clinical examination and thus no baseline clinical characteristics were available.

†Data on neck circumference was available for 42 patients because it was not measured in Offspring exam 8.

‡Upper body subcutaneous neck fat equals total neck fat minus breast fat.

in a community-dwelling sample from the Framingham Heart Study. Second, UBSF is correlated with cardiometabolic risk including strong correlations with other measures of adiposity in our feasibility study.

Figure 2. Intrareader (A) and interreader (B) upper body subcutaneous neck fat measurement.
Although BMI is an excellent indicator of obesity, it does not account for regional fat distribution and cannot differentiate among fat compartments. Fat distribution studies have used imaging modalities such as computed tomography to quantify and differentiate abdominal adipose tissue depots. Both VAT and SAT have been associated with cardiometabolic risk, but VAT has been shown to be a particularly pathogenic fat depot. Data from the Framingham Heart Study showed that waist circumference was highly correlated with VAT (r=0.78 for women and r=0.73 for men) but also with SAT (r=0.87 for women and r=0.88 for men), suggesting that waist circumference cannot discriminate between VAT and SAT. Similar to previous studies of waist circumference, studies of neck circumference have shown an association between UBSF and insulin resistance, elevated blood pressure, and dyslipidemia; however, neck circumference is only a proxy for UBSF. Direct volumetric quantification of this novel depot is needed to understand the full clinical implications.

We have developed a protocol for the quantification of UBSF that has high inter- and intrareader reproducibility. The present study adds a reproducible technique to the literature that allows for further evaluation of upper body fat. Through the use of this protocol, characterization of UBSF can be quantified, leading to the ability to assess the correlations of this fat depot with clinical characteristics and obesity outcomes. Ultimately, implementation of this protocol more broadly may help provide further insight into the role of regional fat distribution and better mechanistic understanding of obesity-related complications related to UBSF.

Major strengths of our study include the use of a large, community-dwelling population with comprehensively defined clinical characteristics. The use of 2 independent readers helped to establish the reproducibility of our protocol. To our knowledge, there are no other gold standard measurements of UBSF, and that limits our ability to assess measurement of validity. The study sample is predominantly of European ancestry, and generalizability to other ethnicities or populations enriched with UBSF is uncertain.

**Conclusion**

UBSF can be reproducibly quantified using computed tomography in a community-dwelling sample from the Framingham Heart Study. UBSF is correlated with cardiometabolic risk and other measures of adiposity. Quantification of this unique fat depot may add to the mechanistic understanding of regional fat distribution.

**Sources of Funding**

This research was conducted in part using data and resources from the FHS of the National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health and Boston University School of Medicine. This work was partially supported by the NHLBI’s FHS (Contract No. N01-HC-25195). Dr Rosenquist is supported through funding from the Whitaker Cardiovascular Institute (T32 HL007224).

**Disclosures**

None.

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**Table 2. Age- and Sex-Adjusted Pearson Correlation Coefficients for Upper Body Subcutaneous Neck Fat and Cardiometabolic Risk Factors**

<table>
<thead>
<tr>
<th></th>
<th>Neck Circumference*</th>
<th>Upper Body Subcutaneous Neck Fat†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.17 (P=0.28)</td>
<td>0.14 (P=0.19)</td>
</tr>
<tr>
<td>BMI</td>
<td>0.73 (P=0.001)</td>
<td>0.89 (P=0.001)</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.65 (P=0.001)</td>
<td>0.90 (P=0.001)</td>
</tr>
<tr>
<td>Neck circumference</td>
<td>NA</td>
<td>0.75 (P=0.001)</td>
</tr>
<tr>
<td>VAT</td>
<td>0.71 (P=0.001)</td>
<td>0.86 (P=0.001)</td>
</tr>
<tr>
<td>SAT</td>
<td>0.56 (P=0.001)</td>
<td>0.87 (P=0.001)</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>0.39 (P=0.011)</td>
<td>0.46 (P=0.001)</td>
</tr>
<tr>
<td>SBP</td>
<td>0.60 (P=0.001)</td>
<td>0.36 (P=0.001)</td>
</tr>
<tr>
<td>HDL</td>
<td>−0.35 (P=0.028)</td>
<td>−0.29 (P=0.005)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.31 (P=0.046)</td>
<td>0.31 (P=0.003)</td>
</tr>
</tbody>
</table>

BMI, indicates body mass index; HDL, high-density lipoprotein; NA, not available; SAT, subcutaneous adipose tissue; SBP, systolic blood pressure; VAT, visceral adipose tissue.

*Data on neck circumference was available for 42 patients because it was not measured in Offspring exam 8.
†Upper body subcutaneous neck fat equals total neck fat minus breast fat.
Reference


SUPPLEMENTAL MATERIAL

Upper Body Subcutaneous Neck Fat Data Acquisition Protocol

Please contact Caroline S. Fox, MD MPH foxca@nhlbi.nih.gov with questions.

1) When you sit down to read each day, confirm Fat template by clicking on 3D Setting and confirming that the settings in A and B on the screen match 1 and 2 here (150, -120, opacity 1.00).

2) Select scan from the patient ID column
3) Select Lung Recon 50 slice option from the box at the lower left
4) Scroll through scout film until you find the level of the sternal landmark
   a. First slice with “bowtie” shape and full trabecular bone
5) Input slice of sternal landmark and select additional 40 slices superior (i.e. sternal landmark at slice 436, there select slices 436-476)

6) **IF the scan does not go through the full length of the selected neck slices, exclude scan but list the reason for exclusion on data acquisition sheet

7) Click on 3D tab at the top of the screen

8) Select the template tab, then double-click on the FAT 4 icon (image to left will become fuzzy)

9) Select curve. Select axial. Respond “yes” to “do you want to reset the current mask”

10) Starting at the sternal landmark, trace the outline of the mediastinum as shown in the example below.
   a. Trace a smooth circle around the mediastinum
   b. If you are satisfied with the tracing, right click and it will become red
11) Go through the image, reading at slices 0, +10, +20, +30, +40
12) After completing this, scroll through all the slices
   a. The yellow dotted lines are the interpolated slices and these can be changed if there are errors by redrawing over the yellow dotted lines
   b. If you do this, be sure to scroll up and down to assess whether changes have affected images already viewed
13) Once completed, return to the first slice and select “**keep region**” (mediastinal region should become green)
14) Drag icon in **Box A** to **Box B** and click “**Reverse**”
15) Click the **3D** tab at the bottom of the screen
16) Select “**measure**” and then “**volume**”
   a. Obj1 = mediastinum fat
   b. Obj2 = total neck fat
17) Select “**output**” at the top of the screen (if there is a picture there already, be sure to delete it)
18) Select “**capture**” along the bottom of the screen
19) Left click on the image and it should show up in the output screen
20) The image in the output screen should have a red border. Left click the image and border should turn green.
21) Select “save file” and save the file using the actual ID number that automatically shows up in the save file box (please delete the extraneous numbers and avoid retyping the ID) and add the following tail:

**ID_Neck1_Readers Name**

a. Save up to 100 jpps (50 participants) in a given electronic folder, which should be named with the name of the person completing the CT reads and which reads the folder contains (e.g. 0001-0050)

b. Upon completion, electronic folders will be printed off and placed in the keying queue

22) Return to the patient list screen. Click the “Lung Recon 50” option again to reset the slice selection. Input the same slice numbers again.

23) Repeat steps 3-20 but this time draw the marking to exclude breast fat.

a. Follow an outline that starts at the sternum and excludes all breast fat by following the pectoralis muscle in the anterior portion and ending at the site of the natural end of breast.

i. In men and patients with little breast tissue follow the natural curve of the pectoralis muscle to the outside portion of the chest.

b. Exclude other anatomical structures including arms/shoulders if they are seen within the region of interest.

i. Arms can usually be delineated from the thorax by identifying the anterior axillary fold. This appears as a “notch” posterior to the pectoralis major. Drawing a line through the anterior axillary fold to the posterior axillary fold should eliminate the arm from the picture.

c. Complete the circle outside of the body cavity making sure to avoid the grading scale at the bottom of the screen.
24) Save the second read as ID_Neck1_B_Reader’s Name
   a. Obj 1 = Upper body volume without breast fat
   b. Obj 2 = Breast Fat volume

*Of note, ultimately the region of interest is (Neck1)Ob2 – (Neck1_B)Ob2 which will be upper body subcutaneous fat exclusive of breast fat and mediastinal fat depots.

Reference of similar methods used to assess VAT and SAT:

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J Am Heart Assoc. 2014;3:e000979; originally published December 18, 2014; doi: 10.1161/JAHA.114.000979
The Journal of the American Heart Association is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Online ISSN: 2047-9980

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://jaha.ahajournals.org/content/3/6/e000979

Data Supplement (unedited) at:
http://jaha.ahajournals.org/content/suppl/2014/12/19/jah3793.DC1
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