SUPPLEMENTAL MATERIAL

Upper Body Subcutaneous Neck Fat Data Acquisition Protocol

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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).

[Figure 3] W/L, Opacity, and Color settings

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
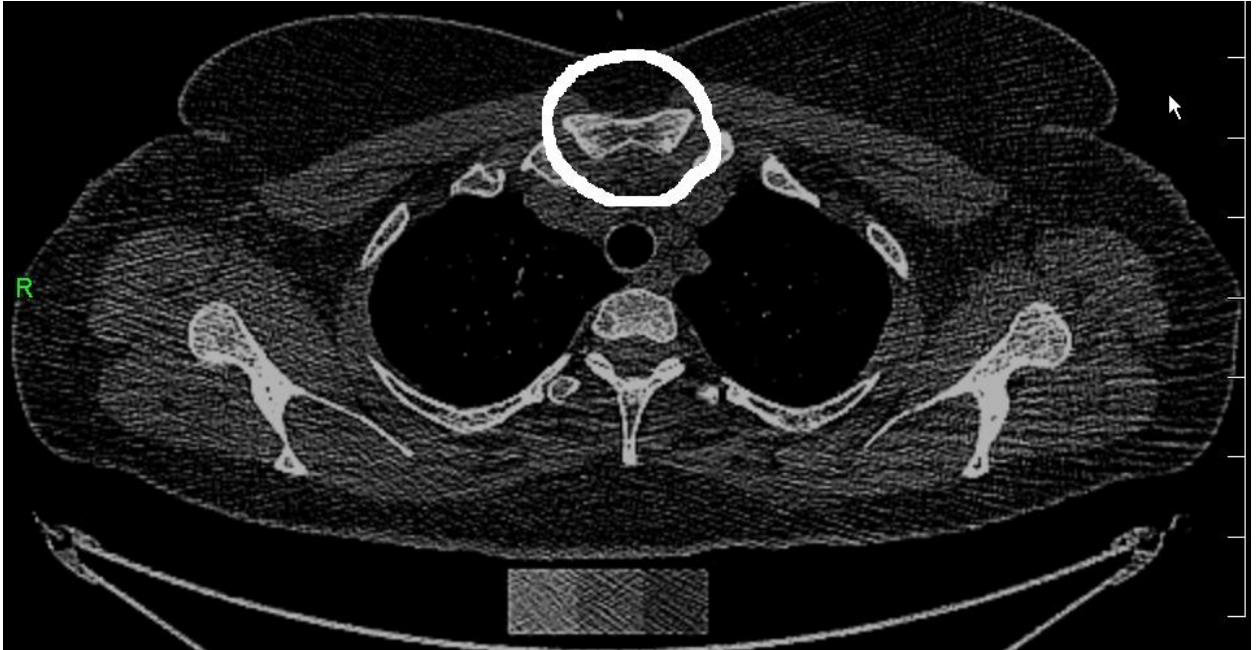
5) Input slice of sternal landmark and select additional 40 slices superior (i.e. sternal landmark at slice 436, there select slices 436-476)
6) **IF the scan does not go through the full length of the selected neck slices, exclude scan but list the reason for exclusion on data acquisition sheet
7) Click 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 jpgs (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:

Maurovich-Horvat P, Massaro J, 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.