

Analysis of Visceral and Subcutaneous Adiposity via Micro-Computed Tomography

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Customer need

To address the etiology and pathophysiology of the current obesity epidemic and to evaluate potential treatments, the precise quantification of adipose tissue is critical. Total adipose tissue (TAT) is not uniformly distributed in the body but, instead, accumulates in specific compartments to become visceral adipose tissue (VAT) or subcutaneous adipose tissue (SAT). General indices of obesity that track TAT, such as the Body Mass Index, do not accurately predict the risk for diabetes and heart disease because the amount of fat in different body compartments carries differential metabolic risks [1]. VAT is more closely correlated with obesity-associated pathologies and complications than either TAT or SAT. Not surprisingly, removing SAT from the abdominal area through liposuction does not provide the health benefits that would be expected

from the reduction in fat mass.



Figure 1: A sagittal (left panel) and coronal (right panel) view of a mouse body scanned by in vivo microcomputed tomography. The skeleton was superimposed upon the adipose tissue (gray) to allow to greater spatial clarity. The abdominal VOI, in which the separation between subcutaneous (yellow) and visceral (red) fat was performed, was defined by precise skeletal landmarks. From [3].

Customer need

Identification and quantification of total, visceral and subcutaneous adipose tissue by in-vivo micro-CT.

Materials and methods

SCANCO Medical vivaCT 40 and vivaCT 75 scanner was used to produce the images. The segmentation algorithms used for the analysis [3] were implemented with SCANCO Image Processing Language (IPL) Software.

Results

It is validated that vivaCT can be used for quantification of discrete fat deposits as well as fat infiltration into organs and tissues of live small rodents. In vivo micro-CT is a non-invasive, quantitative tool that provides a robust, reliable, simple and cost-effective alternative for fat separation with high resolution and selectivity.

In efforts to identify mechanisms and treatments for the health crisis associated with the metabolic syndrome, investigators have increasingly turned to mouse and rat models. *In vivo* imaging techniques have the potential for a more accurate determination of adipose tissue and allow to the tracking of obesity development and treatment efficacy over time. Micro-CT images can not only quantify the volume and geometry of hard tissues, but any tissue with sufficiently large density gradients against the background including discrete fat deposits or infiltration of fat into other organs

can be quantified. As the resolution of *in vivo* micro-CT scans can be selected to fall into an isometric voxel range of approximately ten to two hundred microns, the system can not only measure the total volume of adipose tissue within an animal, but can also identify and quantify small volumes of fat cells residing in discrete deposits. Here we describe a validated method to image and quantify TAT, SAT, and VAT by *in vivo* micro-CT [2, 3].

Materials and methods

Below, the principal steps involved in the *in vivo* quantification of discrete fat deposits by micro-CT are described for small animal models. Scan parameters and the effective use of post-processing data analysis routines that yield precise and accurate data are discussed in greater detail elsewhere [2-4]. All data presented below were generated with the Scanco *in vivo* scanners vivaCT 40 and vivaCT 75. Switzerland).

Energy Settings: When imaging fat, greater efforts have to go into optimizing contrast than when imaging hard tissue. For our scanners, we typically select the highest available current for our source (133 μ A) and a low voltage setting (45 KV). Very small or large animals may require an adjustment of the tube energy parameters and it may take several trials to find the optimal settings.

Resolution: To minimize time and resources necessary to process and store data, the lowest resolution that will provide adequate detail should be selected. Voxel densities of fat are relatively uniform throughout the adipose tissue and partial volume effects are less important than for structures that have an intricate architecture such as trabecular bone. The abdominal muscular wall is commonly used to separate visceral adipose tissue (VAT, inside of the abdominal wall) from subcutaneous adipose tissue (SAT, outside of the abdominal wall). We have found that for many inbred mouse strains, a resolution of approximately 80 μ m is sufficient to accurately identify the muscle fascia of the abdominal wall (Fig. 1).

Region of Interest: Only a whole body scan can determine the amount of total body fat; however, there would be clear advantages in regards to scan time and data storage if similar information could be extracted from scanning a much smaller anatomical region. To this end, we performed whole body scans of forty-five 4mo old C57BL/6J mice across a large range of body mass/adiposity, and compared data from various analysis regions. The largest region spanned almost the entire body from the base of the

skull, as the spinal canal begins to widen, to the distal end of the tibia [2]. The smallest region utilized the same images but the analysis was constricted to the abdominal region between L1 and L5. The large differences in body mass (range: 15.7 - 46.5 grams) were induced by differential diets and a non-pharmacological prophylaxis for adiposity over a 12wk period [5]. Total whole body fat volume was highly correlated with abdominal fat volume across the 45 mice (Fig. 2). These data indicate that scanning an entire mouse may not be necessary to obtain relative data on total body fat as there was no loss in relative information by restricting the region of interest to the abdominal volume between L1 and L5. Reducing the size of the region of interest decreased the scan time from about 35-40 min to 12-13 min.

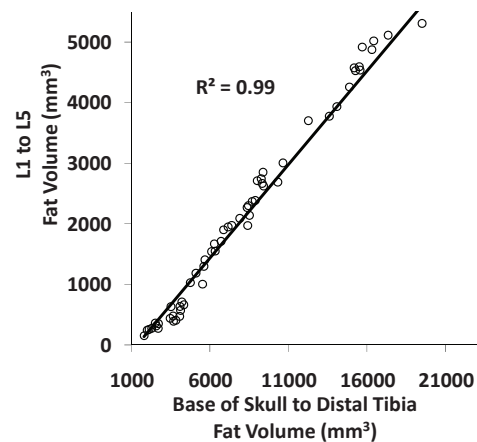


Figure 2: A scan of the abdominal region (L1 to L5) reduced the scan time by two-thirds. Despite the much smaller region, fat volume of the abdomen was highly correlated with total fat volume of the entire mouse body. From [2]

Data Filter and Thresholding: A Gaussian filter is most commonly used to reduce noise in the gray-scale images. In our experience, levels for *sigma* and *support* of 1.0 / 2 have worked well for images scanned at 80 micron resolution, values that should be raised to 1.5 / 3 for 50 micron resolution scans. Upon filtering, thresholding of the image facilitates the segmentation of the tissue of interest from the background.

Automatic Quantification of Discrete Fat Deposits: While the correct interpretation of fat data in metabolism, obesity, and diabetes related research may rely on the detailed volumetric assessment of distinct adipose compartments, it is also clear that such evaluations have to be performed in a precise, accurate, and efficient manner. In CT images, the abdominal muscular wall separating the visceral from the subcutaneous compartment can be used as

the demarcation line for VAT and SAT because of the higher tissue density of muscle [6]. To separate the lower density fat compartments on both sides of the muscular wall, the fascia can be traced manually by drawing contour lines in any given two-dimensional micro-CT slice.

Unfortunately, the manual drawing of contour lines is cumbersome, labor-intensive, and may not yield the desired precision and accuracy. Semi-automated algorithms to separate visceral from subcutaneous fat are much faster but may require the manual definition of a seed point. An automated algorithm that is precise and robust was recently developed to quantify VAT and SAT in micro-CT images [3]. The algorithm is available for download at <http://bme.sunysb.edu/labs/sjudex/miscellaneous.html> and relies on Canny edge detection [7] and mathematical morphological operations to automate the manual contouring process that is otherwise required to spatially delineate the different adipose deposits (Fig. 3).

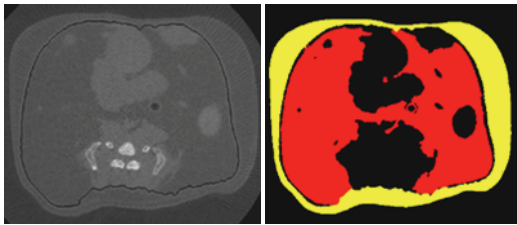


Figure 2: The algorithm automatically detected the abdominal wall (black line, left panel) and then segmented visceral (red) and subcutaneous (yellow) fat (right panel). From [3].

In vivo micro-CT scans of 74 C57BL/6J mice with a broad range of body weights and adiposity were used to test and validate the algorithm [3]. Despite the high heterogeneity within this sample of mice, the algorithm demonstrated a high degree of stability and robustness that did not necessitate changing of any of the initially set input variables. Comparisons of data between the automated and manual methods were in complete agreement ($R^2=0.99$). Robustness was confirmed in a mouse model of severe obesity and in rats. Compared to manual contouring, the increase in precision and accuracy, while decreasing processing time by at least an order of magnitude, suggests that this algorithm can be used effectively to separately assess the development of total, visceral, and subcutaneous adiposity.

Infiltration of Fat into other Organs: Of course, SAT and VAT are not the only fat deposits that can be analyzed. The liver and spleen are two organs whose density values can be measured in a transverse region around the intervertebral disc between the

13th thoracic and first lumbar vertebrae. The inverse liver-to-spleen (L/S) density ratio is an indicator of the degree of fat infiltration in the liver. Using the methods described above, the LS ratio was found to be significantly lower in C57BL/6J mice fed a high-fat diet than in regular diet mice, both at 8mo (52.9%, $p<0.0001$) and 11mo (69.5%, $p<0.0001$) of age [3].

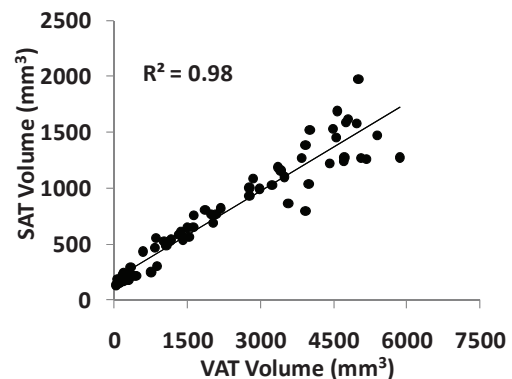
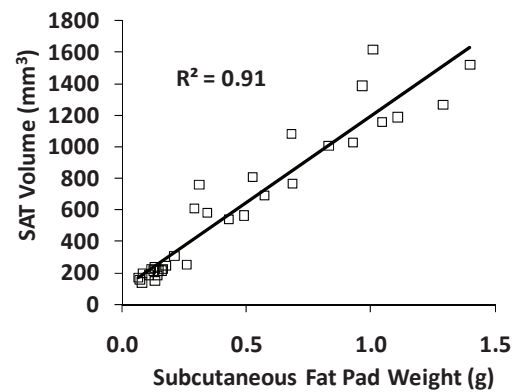
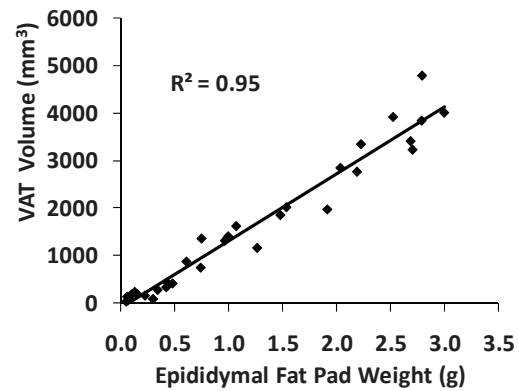


Figure 5. Evaluation of abdominal adiposity separating visceral adipose tissue (VAT) from subcutaneous adipose tissue (SAT). Visceral adipose tissue volume was highly correlated with the weight of the visceral (perigonadal) fat pad ($p<0.001$, top panel). Subcutaneous adipose tissue volume was highly correlated with the weight of the

subcutaneous fat pad ($p < 0.001$, middle panel). As the microCT calculated volumes for both fat deposits correlated well with the weights of the respective fat pads, VAT and SAT area were also correlated to each other ($p < 0.001$, bottom panel). From [2].

Validation of method: While there is little doubt that micro-CT can precisely quantify fat volume based on voxel densities, the technique described above was validated by comparing volumetric micro-CT data to a well established method in the literature. Ninety C57BL/6J mice (weight range: 15.7 - 46.5 grams) were micro-CT scanned *in vivo* at 5 mo of age and subsequently sacrificed. Whole body fat volume (base of skull to distal tibia) derived from *in vivo* micro-CT was significantly ($p < 0.001$) correlated with *ex vivo* tissue weight of discrete perigonadal ($R^2 = 0.94$), and subcutaneous ($R^2 = 0.91$) fat pads. Both the correlations between visceral fat pad weight and micro-CT determined visceral fat volume ($R^2 = 0.95$, $p < 0.001$) as well as subcutaneous fat pad weight and micro-CT determined subcutaneous fat volume ($R^2 = 0.91$, $p < 0.001$) were very high (Fig. 4). The strong correlations exceeded the associations between DXA measurements and fat pad weight [8] and validated *in vivo* micro-CT as a non-invasive, quantitative technique to determine the spatial distribution of specific fat compartments.

Conclusion

A validated method is described that can be used to quantify discrete fat deposits as well as fat infiltration into organs and tissues of live small rodents. Compared to current imaging techniques with similar capabilities, such as microMRI or the combination of DXA with NMR, micro-CT offers higher spatial resolutions and may be more readily available. The high resolution of the method enables the detection of the muscular abdominal wall which is used to separate visceral adiposity from subcutaneous adiposity. An algorithm that standardizes and automates the analysis of the distribution of specific adipose compartments is available. In summary, *in vivo* micro-CT is a non-invasive, quantitative tool that provides a robust, reliable, simple and cost-effective alternative with higher resolution and selectivity than many other methods to precisely determine total and regional adipose volumes and fat infiltration in small rodents.

References

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Numerous references to similar studies can be found on the SCANCO Medical webpage: www.scanco.ch

SCANCO equipment

SCANCO Medical vivaCT 40 and vivaCT 75 scanners.

SCANCO software

Image Processing Language (IPL) including:

- ✓ Automated fat separation