



ORIGINAL ARTICLE

Validity of a new automated software program for visceral adipose tissue estimation

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Introduction: Given the considerable time and research cost of analyzing biomedical images to quantify adipose tissue volumes, automated image analysis methods are highly desirable. Hippo Fat™ is a new software program designed to automatically quantify adipose tissue areas from magnetic resonance images without user inputs. Hippo Fat™ has yet to be independently validated against commonly used image analysis software programs.

Objective: Our aim was to compare estimates of VAT (visceral adipose tissue) and SAT (subcutaneous adipose tissue) using the new Hippo Fat™ software against those from a widely used, validated, computer-assisted manual method (*slice-O-matic* version 4.2, Tomovision, Montreal, CA, USA) to assess its potential utility for large-scale studies.

Methods: A Siemens Magnetom Vision 1.5-T whole-body scanner and a T1-weighted fast-spin echo pulse sequence were used to collect multiple, contiguous axial images of the abdomen from a sample of 40 healthy adults (20 men) aged 18–77 years of age, with mean body mass index of 29 kg/m² (range = 19–43 kg/m²).

Results: Hippo Fat™ provided estimates of VAT and SAT that were highly correlated with estimates using *slice-O-matic* ($R^2 > 0.9$). Average VAT was 9.4% lower and average SAT was 3.7% higher using Hippo Fat™ compared to *slice-O-matic*; the overestimation of SAT tended to be greater among individuals with greater adiposity. Individual-level differences for VAT were also substantial; Hippo Fat™ gave estimates of VAT ranging from 1184 cm³ less to 566 cm³ more than estimates for the same person using *slice-O-matic*.

Conclusion: Hippo Fat™ provides a rapid method of quantifying total VAT, although the method does not provide estimates that are interchangeable with *slice-O-matic* at either the group (mean) or individual level.

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Introduction

It has become apparent in recent years that the metabolic and hormonal function of adipose tissue is not uniform, but instead varies from site to site in the body. Visceral adipose tissue (VAT) (adipose tissue deposited around the internal organs of the abdomen, trunk and pelvis) appears to hold particular risk for the metabolic syndrome and cardiovascular disease (CVD).^{1–10} This increased risk may operate

though the association between VAT and vascular inflammation, hormonal variations and insulin resistance.

Safe and cost-effective methods of quantifying internal adipose tissue mass such as VAT are needed, as an increasing number of pharmaceuticals are being targeted toward adipose tissue reduction,¹¹ whereas others, such as anti-depressive and anti-psychotic medications, have known side effects on adipose tissue mass and distribution.¹² It is therefore increasingly desirable to monitor changes in specific adipose tissue compartments in clinical studies. Both computed tomography (CT) and magnetic resonance imaging (MRI) are well-established methods for the estimation of VAT mass,¹³ whereas proton magnetic resonance spectroscopy (¹H MRS) has emerged as a useful non-invasive method of quantifying lipid depositions within hepatic and skeletal muscle cells *in vivo*.^{14,15} CT provides high-resolution

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images that facilitate image segmentation, but it necessitates exposure of subjects to ionizing radiation. Without ionizing radiation, MRI provides a safer alternative for adipose tissue quantification that has excellent agreement with adipose tissue values produced by dissection and chemical analysis.^{16,17} A number of commercial and freeware biomedical image analysis software packages are available for segmenting MR images, each of which differs in the algorithms used to identify and measure anatomical structures, as well as in their flexibility, time efficiency and ease of use. Popular biomedical image analysis software packages include *Analyze* (Mayo Clinic, Rochester, MN, USA), *slice-O-matic*[™] (Tomovision, Montreal, Canada) and the public domain programs *NIH Image and ImageJ* (National Institutes of Health, Bethesda, MD, USA).

Positano *et al.*¹⁸ recently introduced Hippo Fat[™], an IDL Virtual Machine 6.1-based freeware designed to quantify adipose tissue areas from MR images automatically, without user supervision or manual editing.¹⁹ Given the considerable time and research cost associated with manual tagging of VAT, an automated image analysis system is highly desirable, especially for large-scale studies. Compared to a free-hand method of contour fitting,²⁰ Hippo Fat[™] produced comparable results in less time,¹⁸ but Hippo Fat[™] has yet to be independently compared to image analysis software currently in use in clinical and epidemiological studies. *slice-O-matic*[™] (*slice-O-matic version 4.2*, Tomovision, Montreal, Canada) has been used to estimate visceral adipose tissue volumes in over 200 peer-reviewed scientific publications since 1991.^{21–26} It has been validated against chemical analysis and other image analysis methods^{21,27} and has been used as a reference standard method for comparison in several methodological reports.^{28–30} The goal of this study was to compare the reliability and the validity of the new Hippo Fat[™] software against *slice-O-matic*[™] software to assess its utility in measuring visceral and subcutaneous adipose tissues in epidemiological studies.

Materials and methods

Subjects

Forty healthy subjects (20 men; 20 women) aged 18–77 years were randomly selected from those enrolled in the Southwest Ohio Family Study for participation in this validation study. The Southwest Ohio Family Study aims to identify genomic regions influencing CVD risk factors, including body composition and inflammation, in five large extended families ascertained on a male proband with diastolic blood pressure over 95 mm Hg. Participants for this sub-study were pre-screened to insure they were free of any contraindications for MRI (including pacemakers and metal implants). The study protocols and informed consent documents were approved by the Wright State University Institutional Review Board before subject participation.

Acquisition of MR images

MR images were collected at the Good Samaritan Hospital Greater Dayton MRI Consortium in Dayton, Ohio. Subjects were instructed to lie in the magnet in a supine position with arms extended above the head. Images were obtained with a Siemens Magnetom Vision 1.5-T whole-body scanner using a T1-weighted fast-spin echo pulse sequence (TR 322 ms, TE 12 ms). A breath-hold sequence (approximately 22 s/acquisition) was used to minimize the effects of respiratory motion on the images. All images were acquired on a 256 × 256 mm matrix, with a rectangular 480 × 360 mm field of view. Slice thickness was 1 cm and contiguous slices were obtained every 1 cm from the ninth thoracic vertebra (T9) to the first sacral vertebra (S1). Depending on the height of the person, this resulted in a range of 21–35 MRI axial images per person. The images were retrieved from the scanner using the DICOM (Digital Imaging and Communications in Medicine) protocol (National Electrical Manufacturer's Association, Rosslyn, VA, USA). We begin tagging adipose tissue from the first image that contains the upper margin of the liver down to the L5–S1 image, which increases the likelihood that all intra-abdominal adipose tissue will be included. It should be borne in mind that the anatomic region covered by these images also includes lower mediastinal and pericardial adipose tissue, so that these thoracic depots are also, for the purposes of this study, classified as abdominal visceral adipose tissue.

Slice-O-matic analysis

Image analyses for this study were performed by a single trained observer. All raw MR images between T9 and S1 were opened in *slice-O-matic*[™] version 4.2 and sorted into anatomical order; tagging was initiated at the image containing the upper margin of the liver and continued down to the image containing the L5–S1 intervertebral space. To maximize contrast and maintain consistency, the *slice-O-matic*[™] 'Color Scheme' or brightness settings were set at zero for the lowest signal intensity level and at 1278 for the highest signal intensity level for the gray-scale images. Both SAT and VAT were tagged by using the program's 'Region Growing/Painting' mode. Within the 'Region growing/Painting' control panel, the upper threshold was set to the maximum possible setting and the lower threshold was set to a value within the range of 350–650, with the exact value dependent on the pixel intensity of the SAT and VAT in the particular series of images. Each consecutive slice was tagged using the same region growing procedure. SAT and VAT tags resulting from the 'Region Growing/Painting' mode were then manually inspected and edited as needed in the 'Edit' mode to identify image artifacts and to manually tag adipose tissue not previously tagged (adipose tissue areas below the chosen threshold). Intra-observer reliability for estimation of total VAT volume using *slice-O-matic*[™] is good, and similar to previously reported values (coefficient of variation (CV) = 4.5%; intraclass correlation (ICC) = 0.997)²² (Table 1), and there was no

significant variation in intra-observer reliability depending on which particular image was compared.

Hippo Fat™ analysis

Hippo Fat™ version 6.0 uses a fuzzy c-mean (FCM) algorithm to compute a measure of membership (the fuzzy membership) at each pixel for each of three tissue classes (air, adipose tissue and non-adipose tissue).^{18,31} The FCM algorithm results in a roughly bimodal distribution of pixel intensities in the visceral compartment for each axial image. A Gaussian distribution (with a mean value equal to the representative adipose tissue value and a height equal to the number of pixels with that range of values) is then fit to the peak that corresponds to adipose tissue to estimate SAT and VAT areas. Three contour lines are then automatically generated for each image: (1) along the outer margin of the SAT, (2) along the inner margin of the SAT and (3) around the smallest possible region in the visceral region that includes all VAT. A histogram showing the distribution of pixel intensity values within the SAT and VAT contours is then automatically generated for each image, and the upper (adipose tissue-specific) peak for each is identified and a Gaussian curve automatically fit to the peak, allowing the adipose tissue area to be calculated. In our case, the analyst then manually adjusted both the contour lines and the shape of Gaussian curve by eye, as necessary, using the edit function. The analyst used Hippo Fat™ to conduct repeated SAT and VAT analyses for a set of $N = 10$ subjects to calculate intra-observer reliability. The 2 observation days were separated by at least 2 weeks, and the observer was blinded to the results from day 1 before re-running the program on day 2. Intra-observer reliability for the estimation of VAT using Hippo Fat™ was good ($CV = 7.25\%$; $ICC = 0.991$), but lower than for slice-O-matic (Table 1). Intra-observer reliability tended to be somewhat better for images that were higher in the abdomen ($CV = 3\text{--}8\%$) than for images lower in the abdomen ($CV = 11\%$) (data not shown).

Statistical methods

Means and s.d. were used to describe the study sample. The ICC was used to determine the strength of association between VAT and SAT estimates from readings 1 and 2 to

estimate the intra-observer reliability of each method. To quantify the measurement error of each method, the CV and the technical error of measurement (TEM) were used. A paired Student's *t*-test was used to detect differences in mean VAT and mean SAT using slice-O-matic and Hippo Fat™. The agreement between the methods was ascertained by linear regression analysis, with VAT (or SAT) from Hippo Fat™ as the dependent variable, and with VAT (or SAT) from slice-O-matic as the independent variable. Simultaneous tests were conducted to determine if the slopes of the fitted regression lines were significantly different from 1, and if the intercepts were significantly different from 0. Bland-Altman plots were used to examine the relationship, if any, between the inter-method differences in VAT and SAT and the average level of VAT and SAT, respectively, and to examine the 95% confidence intervals of the individual-level agreement between the two methods. Volumetric units were used throughout (cm^3), except where mass was calculated (using the conversion equation of 1 adipose tissue = 0.923 kg) in order to illustrate the inter-method differences in units that are more familiar in the context of obesity and weight loss.

All analyses were conducted using SAS, version 9.1 (SAS Institute, Cary, NC, USA) and results were considered statistically significant at $P < 0.05$.

Results

Descriptive statistics of the study population are presented in Table 2. The participants in the study were overweight on average (body mass index (BMI) = 28 kg/m^2 in men and 29 kg/m^2 in women), and BMI ranged from 19 to 43 kg/m^2 . Using the average of the SAT and VAT values from the two analysis methods, women tended to have higher SAT ($P = 0.09$) and lower VAT ($P = 0.004$) than men.

Agreement between the two analysis methods was very good; R^2 values were 0.9275 for VAT and 0.9989 for SAT (sexes combined) (Table 3). Adjusting for age and BMI did not substantially change the R^2 values (data not shown). R^2 was substantially lower in males (0.93 for VAT and 0.91 for SAT) than females (0.99 for VAT and 0.99 for SAT). Despite the relatively strong correlation between the two methods, Hippo Fat™ resulted in significantly lower mean estimates of VAT (2989.6 ± 1492.0 vs $3298.7 \pm 1632.2 \text{ cm}^3$, $P < 0.0001$,

Table 1 Intra-observer reliabilities of SAT and VAT volume estimated from slice-O-matic and Hippo Fat™

	N (pairs)	Reading 1 (mean, cm^3)	Reading 2 (mean, cm^3)	Difference (cm^3)	s.d.	TEM (cm^3)	CV (%)	ICC
<i>Slice-O-matic</i>								
SAT	10	5040.00	5146.41	106.59	84.08	94.14	1.85	0.9996
VAT	10	3112.13	3195.36	151.28	141.40	142.97	4.53	0.9967
<i>Hippo Fat</i>								
SAT	10	5261.46	5201.94	81.88	108.13	92.81	1.77	0.9996
VAT	10	2550.81	2677.73	190.05	199.49	189.65	7.25	0.9914

Abbreviations: CV, coefficient of variation; difference, mean difference between repeated readings by the same observer; ICC, intraclass correlation coefficient; SAT, abdominal subcutaneous adipose tissue; s.d., standard deviation; TEM, technical error of measurement; VAT, abdominal visceral adipose tissue.

Table 2 Description of study sample (Mean \pm s.d., range)

	Males (N = 20)	Females (N = 20)
Age (years)	46.4 \pm 13.9 (22.4–69.2)	49.6 \pm 13.4 (18.2–77.4)
Height (cm)	179.6 \pm 5.3 (171.2–191.7)	161.8 \pm 8.0 (147.9–174.0) *
Weight (kg)	91.3 \pm 13.6 (69.5–118.2)	77.1 \pm 18.2 (54.9–127.2) *
BMI (kg/m ²)	28.3 \pm 4.0 (22.8–36.9)	29.4 \pm 6.0 (19.4–43.0)
Average SAT (cm ³)	4841 \pm 2550 (1572–10 133)	6120 \pm 3032 (1285–13 392)
Average VAT (cm ³)	3617 \pm 1613 (513–6824)	2671 \pm 1358 (204–4582) *

Abbreviations: BMI, body mass index; average SAT, average subcutaneous adipose tissue for two compared methods; average VAT, average visceral adipose tissue for two compared methods. Sex difference significant at $P < 0.05$.

Table 3 Agreement between Hippo Fat and slice-O-matic in the estimation of SAT and VAT

	Intercept (s.e.)	Slope (s.e.)	R ²
VAT			
Males	114.0 (241.4)	0.87 (0.06)*	0.9260
Females	53.2 (202.5)*	0.89 (0.06)	0.9984
Sexes combined	85.5 (146.5)	0.88 (0.04)**	0.9275
SAT			
Males	122.4 (50.5)	1.01 (0.01)*	0.9130
Females	145.3 (40.6)**	1.01 (0.01)*	0.9994
Sexes combined	120.0 (32.9)**	1.01 (0.01)**	0.9989

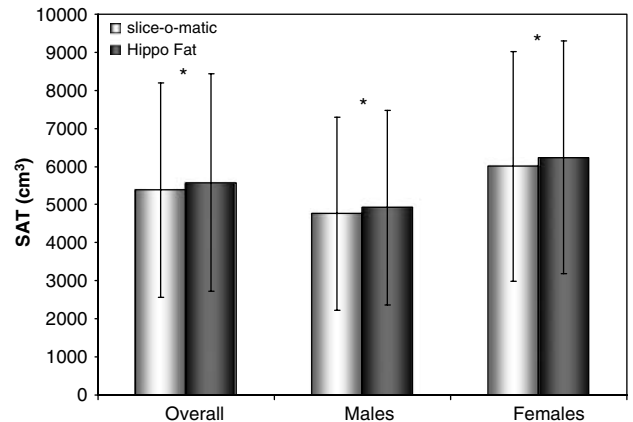
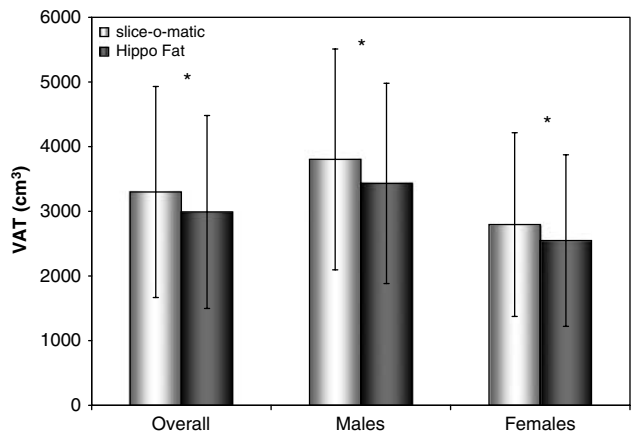
Abbreviations: s.e. = standard error. * $P < 0.05$; ** $P < 0.01$; P -values refer to significance of the difference between the estimate and 0.0 (in the case of the intercept) and between the estimate and 1.0 (in the case of the slope).

sexes combined) and significantly higher mean estimates of SAT (5579.5 ± 2861.2 vs 5382.7 ± 2819.4 cm³, $P < 0.0001$, sexes combined) compared to slice-O-matic™ (see Figures 1 and 2). At the group level, therefore, the average inter-method difference in VAT was 9.4% and the average inter-method difference in SAT was 3.7%. The mean inter-method differences did not vary by sex. We examined inter-method agreement for mean SAT and VAT volume at the L4–L5 image to test if inter-method differences might depend on which abdominal location was chosen for comparison. Agreement was somewhat better for the L4–L5 image alone (VAT underestimated by 7.8%; SAT overestimated by 1.5%) as compared to the agreement for all images combined.

Bland–Altman plots show that as the mean SAT level increased, the differences between the methods increased ($r = 0.41$, $P = 0.009$) (Figure 3); inter-method differences in VAT did not vary significantly with VAT level (Figure 4). At the individual level, the 95% confidence intervals for the prediction of slice-O-matic™ adipose tissue volumes from Hippo Fat™ volumes ranged from -4 to $+398$ cm³ for SAT, and from -1184 cm³ to $+566$ cm³ for VAT.

Discussion

In this sample of healthy adults aged 18–77 years of age, estimates of visceral adipose tissue volume using the recently introduced Hippo Fat™ program were highly correlated with

**Figure 1** Mean (s.d.) abdominal SAT: Hippo Fat™ vs slice-O-matic (* $P < 0.05$).**Figure 2** Mean (s.d.) abdominal VAT: Hippo Fat™ vs slice-O-matic (* $P < 0.05$).

estimates using slice-O-matic™, a semiautomated image segmentation program widely used to measure visceral adiposity^{25,29,32–35} that has been validated against directly measured chemical composition.²⁷ Although there was good overall agreement between the methods ($R^2 > 0.9$ and slope of nearly 1.0), they produced significantly different estimates of SAT and VAT at both the group and the individual levels. Average VAT was underestimated by 9.4% and average SAT was overestimated by 3.7% using Hippo Fat™ compared to

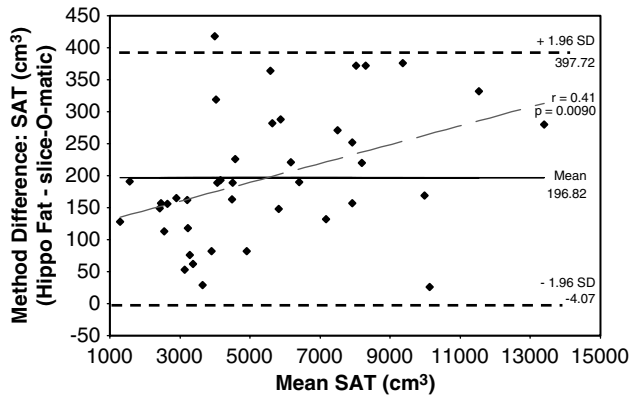


Figure 3 Bland-Altman plot: abdominal SAT differences, hippo Fat™ vs slice-O-matic.

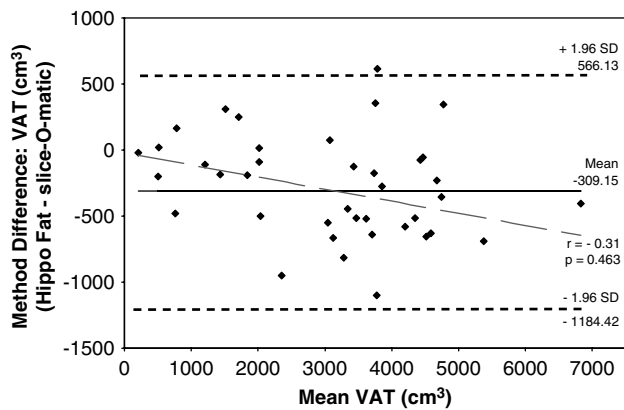


Figure 4 Bland-Altman plot: abdominal VAT differences, Hippo Fat™ vs slice-O-matic.

slice-O-matic™. The overestimation of SAT tended to be greater among individuals with higher SAT levels. Converting the volumetric units to mass, the inter-method difference in SAT was 0.18 kg and the difference in VAT was 0.38 kg. Individual-level error for VAT was also substantial; Hippo Fat™ gave estimates of VAT ranging from 1.07 kg less to 0.51 kg more than estimates from the same person using slice-O-matic™. Therefore, slice-O-matic™ and Hippo Fat™ do not provide interchangeable results for total abdominal VAT or SAT volume. When designing a clinical or epidemiological study, the same image analysis method should be used for all observations and at all study centers. This would be particularly important for longitudinal and intervention studies where a change in image analysis software from baseline to follow-up could substantially bias the results for or against an effect on visceral adiposity.

While we did not examine the agreement between the methods on a slice-by-slice basis, it is possible that agreement may be greater or lesser at specific individual slices or when examining particular adipose tissue depots (e.g., mediastinal, pericardial, retroperitoneal). We found, for

example, that for the image taken at the L4–L5 intervertebral space, the single image location most commonly used for MRI studies of visceral adiposity, the degree of overestimation of SAT area and the degree of underestimation of VAT area were lower than we found for total SAT and VAT volumes.

Hippo Fat™ software can be run without user supervision or manual editing, and can process 32 images from a single subject in approximately 6 min. This compares very favorably to the 60 min required to process the same number of images using slice-O-matic™. However, when we first attempted to use Hippo Fat™ without performing manual editing, the inter-method difference was ~19% for VAT and ~10% for SAT (data not presented). We therefore took advantage of the manual editing features of Hippo Fat™ to adjust both the VAT contour and the Gaussian distribution settings, which increased the processing time to 30 min per individual having a set of ~28 MR images. In the case of individuals with low levels of adipose tissue, the non-adipose tissue and the adipose tissue peaks in the histogram somewhat overlapped one another, creating the need to edit the Gaussian curves manually. This issue has been noted previously.¹⁸

Even in the case of heavier subjects with large amounts of visceral adipose tissue, the automated contours for the visceral compartment appeared to exclude some of the adipose tissue from the subsequent analysis, perhaps because of varying intensity of the VAT signal across a single image, or poor resolution of the boundary between the abdominal wall and the visceral compartment. If the signal intensity for VAT is low in a region, the automated VAT contour may not detect it, and the VAT level may be underestimated. Image quality may therefore determine if Hippo Fat™ can be used successfully in its completely automated mode, or if manual adjustments will be required. Subjects in the present analysis were imaged on a 1.5T scanner using a standard body coil with a T1-weighted fast-spin-echo pulse sequence – a fairly standard protocol for the acquisition of abdominal VAT data in existing studies and similar to the protocol used to initially test the Hippo Fat™ method.¹⁸ Better resolution images may improve the ability of Hippo Fat™ to generate accurate estimates of VAT without manual editing.

A disadvantage of the Hippo Fat™ program is that it is designed to estimate the total amount of SAT and VAT in the abdominal region only. Recent work has established that there are sub-compartments of adipose tissue within both the subcutaneous compartment, including deep and superficial adipose tissue,^{36,37} as well as the visceral adipose tissue compartment, including pericardial adipose tissue, mediastinal adipose tissue and others³⁸ that may play particularly important roles in the development of disease.³⁹ Computer-assisted manual editing programs such as slice-O-matic™ allow particular adipose tissue sub-compartments to be visually identified and tagged and therefore hold a key advantage for flexible body composition assessment.

In conclusion, there was good overall agreement between Hippo Fat™, a newly introduced automated method for measuring visceral adipose tissue in humans, and a widely used, validated semiautomated software. However, the two methods produced significantly different estimates of SAT and VAT at both the group and the individual levels and are therefore not interchangeable. When designing a clinical or epidemiological study, the same image analysis method should be used for all observations and at all study centers. Advantages of Hippo Fat™ are low cost and a significant reduction in the time required for image analyses, which becomes particularly important in studies where large numbers of participants and/or large numbers of images per participant must be analyzed. Disadvantages of Hippo Fat™ are that the program does not allow for measurement of sub-compartments within the subcutaneous and visceral adipose tissue depots and that manual editing may be required, unless the MR images being used have excellent resolution and quality.

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