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Calibration of a semi-automated segmenting method for quantification of adipose tissue compartments from magnetic resonance images of mice

Philippe Garteiser^{1,‡}, Sabrina Doblas^{1,‡}, Rheal A. Towner^{1,*}, and Timothy M. Griffin^{2,3}

¹Advanced Magnetic Resonance Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104

²Free Radical Biology & Aging, Oklahoma Medical Research Foundation, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104

³Department of Biochemistry and Molecular Biology and Department of Geriatric Medicine, Reynolds Oklahoma Center on Aging, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104

Abstract

Objective—To use an automated water-suppressed magnetic resonance imaging (MRI) method to objectively assess adipose tissue (AT) volumes in whole body and specific regional body components (subcutaneous, thoracic and peritoneal) of obese and lean mice.

Materials/Methods—Water-suppressed MRI were obtained on a 7 Tesla, horizontal-bore MRI system in whole bodies (excluding head) of 26 wk old male C57BL6J mice fed a control (10% kcal fat) or high-fat diet (60% kcal fat) for 20 wks. Manual (outlined regions) versus automated (Gaussian fitting applied to threshold-weighted images) segmentation procedures were compared for whole body AT and regional AT volumes (i.e., subcutaneous, thoracic, and peritoneal). The AT automated segmentation method was compared to dual-energy X-ray (DXA) analysis.

Results—The average AT volumes for whole body and individual compartments correlated well between the manual outlining and the automated methods ($R^2 > 0.77$, $p < 0.05$). Subcutaneous, peritoneal, and total body AT volumes were increased 2–3 fold and thoracic AT volume increased more than 5-fold in diet-induced obese mice versus controls ($p < 0.05$). MRI and DXA-based method comparisons were highly correlative ($R^2 = 0.94$, $p < 0.0001$).

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*Corresponding author: Rheal A. Towner, Advanced Magnetic Resonance Center, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104; Phone: (405) 271-7383; Rheal-Towner@omrf.org.

‡Present address: P. Garteiser and S. Doblas, Centre de Recherche Biomédicale Bichat-Beaujon, INSERM U773, Clichy, France

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Author Contributions: TMG was the originator of the idea to conduct body fat analysis on obese and lean mice using an MRI approach, contributed towards data analysis, conducted the DXA study, and contributed towards the writing of the manuscript. PG, SD and RAT all contributed towards the MRI methods development. PG and SD obtained the MRI data and both contributed to data analysis of the MRI study. PG developed the Mathematica notebook to analyze the MRI data using the Gaussian fitted automated segmentation method. Both PG and SD contributed towards writing of the manuscript. RAT contributed towards interpretation of the MRI data, and writing of the manuscript.

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Conclusions—Automated AT segmentation of water-suppressed MRI data using a global Gaussian filtering algorithm resulted in a fairly accurate assessment of total and regional AT volumes in a preclinical mouse model of obesity.

Keywords

Body fat distribution analysis; fat-weighted MRI; diet-induced obesity; adiposity

1. Introduction

Numerous metabolic disease conditions are associated with changes in the quantity and distribution of adipose tissue (AT) throughout the body. Magnetic resonance imaging (MRI) is a highly effective and validated technique for quantifying anatomically distinct AT volumes in both animal and clinical studies [1–3]. Many MRI techniques readily implemented for AT quantification (T1-weighting, selective saturation or chemical shift approaches) are based on contrast difference between water and fat. One recurrent challenge is thus the selection of a method able to process image contrasts to identify and quantify AT volumes in an accurate, efficient, and cost- and time-effective manner.

Calculating AT volumes can be carried out using a variety of approaches to select AT pixels, including manual outline methods, connectivity-based tools, and threshold-based methods. AT volume is then calculated using individual pixel size and the number of selected pixels. Manual approaches consist of outlining AT pixels by hand, by combining anatomical knowledge and image contrast to place boundaries of AT areas on the image with a conventional interface device such as a mouse. A variety of computer graphics programs offer a free hand tool, which enables this type of analysis with relative ease. These methods are simple to implement but are prone to variability because of their inherent subjectivity [4]. They also rely on the parameters of a lookup table used for displaying pixel intensity values. These two disadvantages can be mitigated by blinding the operators to the identification of the images and by merging the results obtained from multiple operators. This comes at the cost of significantly longer analyses, which is not always possible, affordable or practical.

At the other end of the image processing spectrum, connectivity-based tools combine several criteria to determine whether a pixel needs to be considered as belonging to AT, which always include the application of a threshold criterion combined to a neighboring pixel intensity analysis. As such, connectivity-based tools have the potential to be more robust than other approaches, but their reliance on the contiguity criterion makes them sensitive to the morphological topology of some AT deposits. Consequently, they invariably require some degree of user input. An efficient and time-effective alternative approach is a threshold-based method, where an intensity threshold value is determined and pixels above this value can be tallied as AT. Pixel intensity thresholding methods are much less time-consuming than the manual outlining method and much more easily implemented than connectivity-based tools. However, the proper selection of a threshold may be problematic it should be objective while providing robust AT volume measurement from one acquisition to another and within one dataset. Fixing a constant threshold is not ideal because image intensity can vary from one region of the image to another due to radiofrequency field inhomogeneities, receive coil sensitivity profile and main magnetic field inhomogeneities or imperfect properties of the pulse sequences. Such inconsistency can result in significantly different AT pixel intensities for different AT locations within or between slices from a given subject or between subjects.

Here we present a new Gaussian fit approach to provide an automated thresholding method that accounts for image intensity inhomogeneity. We applied this technique to compute whole-body and body compartment-specific AT volume from water-suppressed MR images obtained from high-fat diet-induced obese C57BL6J mice and their dietary controls. We evaluated this technique by comparison to manual selection of whole-body and site-specific AT volume, and to dual-energy X-ray absorptiometry (DXA) measurements of fat mass.

2. Materials and Methods

2.1. Animals

All experiments were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee at the Oklahoma Medical Research Foundation. 20 wk old male C57BL6 mice were purchased from the JAX Diet-Induced Obesity Model service (Jackson Laboratory, Bar Harbor, ME). Mice were fed either a 10% kcal fat diet (D12450B; Research Diets Inc., New Brunswick, NJ) or a 60% kcal fat diet (D12492, Research Diets Inc.) beginning at 6 wks of age. Animals were maintained on their respective diet while housed in a temperature-controlled room on 12-hr light/dark cycles with ad libitum access to food and water. Animals (39 mice; 20 lean and 19 obese) underwent age-matched MRI and DXA scans between 22–36 wks of age. Only mice that maintained body mass within 5% at each timepoint were used for the comparison between MRI and DXA analysis, resulting in 32 mice being used (N=14 lean and N=16 obese).

2.2. MRI acquisition

MR images were acquired on a 7 Tesla (T), 30 cm horizontal bore USR Bruker system equipped with an AVANCE I console. A quadrature coil of 150 mm inner diameter and 266 mm in length matched and tuned to 300 MHz was used for pulse transmission and signal detection. The dimensions of the coil used were chosen to be much larger than the typical body size of the subjects in an effort to minimize edge effects, which may result in important image artifacts that require advanced post-processing manipulations. After conventional scout scans for animal positioning, spin-echo scans were acquired using a repetition time of 1300 ms and an echo time of 15 ms. This parameter set yielded an image contrast in which AT deposits were bright. The geometry used was 25 contiguous horizontal slices of 1mm thickness with a 70 mm × 40 mm field of view encoded over a 384×256 matrix. Inclusive slices were obtained from dorsal to ventral body positions from the base of the skull to the base of the tail. Read direction was along the rostro-caudal axis of the animal, with data sampled over a 50kHz bandwidth. Two such scans were acquired, differing by the presence of a frequency-selective gaussian saturation pulse of 450 Hz in bandwidth and centered on the water frequency before the imaging train, yielding a “water-suppressed” image in which the contrast between AT regions and regions having a typically low fat content (such as muscle or brain) was even higher. To validate the spectral selectivity of this saturation scheme, a spin-echo acquisition was conducted using the same parameter set as described above on tubes of deionized water and olive oil (5 mm diameter, 50 mm length). Average signal intensity within these tubes was calculated for both normal and water-suppressed images.

2.3. Manual segmenting procedure

Regions of interest (ROI) corresponding to high-intensity pixels attributed to AT were generated using the ROI tool of the Paravision software (Bruker). AT deposits corresponding to the subcutaneous, intrathoracic (i.e., thoracic), and intra-abdominopelvic (i.e., peritoneal) AT compartments [2] were manually outlined and itemized on each slice of each animal by three independent operators (N=3 mice per diet). Operators were asked to include compartment attribution data in their measures, in which each ROI was attributed to

either the subcutaneous, thoracic, or peritoneal AT compartments. High intensity pixels attributed to AT were occasionally observed within the liver; however, these pixels were intentionally omitted from the segmentation. AT segmentation was conducted using the water-suppressed datasets. Non-water-suppressed datasets (i.e., regular images) were used to identify anatomical landmarks for segmenting total body and body compartment specific volumes. The volumes of AT for each compartment for each slice were calculated by summing the areas of each ROI and multiplying the result by the slice thickness. As a time-saving measure, this last step was done using a purpose-built python script driving Paravision's ROI tool interface. Total compartment volumes were obtained by summing the compartment volumes calculated on each slice from the non-water-suppressed images. Total body volumes were calculated by adding the volumes of all available total body outlines. For statistical analyses, AT volumes in the different compartments were then expressed as a percent of the total body volume.

2.4. Gaussian fitting of the pixel intensity histograms

A Mathematica (version 6.0; Wolfram Research, Champaign, IL, USA) notebook was created by our group for implementing the automated segmentation method. The water suppressed images were used to generate pixel intensity histograms in which the total available dynamic range was distributed linearly across 1000 intensity bins of equal widths. Two peaks were easily visible in these histograms, with the high intensity peak (typically bins 400 to 700 for water suppressed images) corresponding to the fat areas and the low intensity peak consisting of the background noise. The high intensity peak was numerically fitted to a Gaussian model:

$$y = A \cdot \exp[-(x - \mu)^2 / 2\sigma^2] / \sigma \sqrt{2\pi} \quad (1)$$

where y is the number of pixels in the x -th intensity bin, A is a global scale factor, and μ and σ are the mean and standard deviation of the distribution, respectively. The maximum of the histogram in the 400 to 700 interval was used as an initial guess for the mean. To decrease computing time, the numerical fitting procedure was guided by constraining the median to a 100 bin wide interval centered on the initial guess, the scale factor to less than 2000, and the standard deviation to a 10 to 200 bin interval. On rare occasions, the standard deviation parameters found by the numerical fit were equal to the upper bound of the constraint interval, indicative of a failure of the fitting procedure. This mostly occurred in slices that did not intersect the animal, which were dismissed from further analysis. On each slice of each animal, the average and standard deviations resulting from the fit were then converted back into the pixel intensity scale and stored for further analysis.

2.5. Automated segmenting procedure

Images were segmented by applying a simple threshold criterion for determining the nature (AT or non AT) of each pixel. The mean and standard deviations obtained from the Gaussian fits on each slice were used in the calculation of pixel intensity thresholds. Two strategies were employed. In the first method, threshold values were calculated on a per slice basis as

$$threshold_{slice} = \mu_{slice} - n \cdot \sigma_{slice}, \quad (2)$$

where μ_{slice} and σ_{slice} are the mean and standard deviation obtained from the numerical fit procedure on a given slice, respectively. Segmenting was performed at three different levels, corresponding to values of 1, 2 or 3 for n . In the second method, all determined μ_{slice} and σ_{slice} were averaged to provide a single global value for the mean (μ_{global}) and the standard

deviation (σ_{global}) of the fits. Similarly, these global values were used for the purpose of segmenting images using

$$threshold_{global} = \mu_{global} - n \cdot \sigma_{global} \quad (3)$$

at three different levels of n ($n=1, 2$ or 3).

2.6. Segmenting procedure for compartment analyses

The segmentation of AT was also carried out on subcutaneous, peritoneal and thoracic compartments. Operators were asked to outline the peritoneal cavity and the thoracic cavity. The diaphragm was used as demarcation line between these body cavities. The threshold-based segmentation was carried out on cutaways of these manually drawn compartments. Subcutaneous AT volumes were computed as the difference between total AT volume and the sum of peritoneal and thoracic AT volumes.

2.7. DXA analysis

Total body fat content was measured under anesthesia using a dual-energy X-ray absorptiometry system (Lunar PIXImus2; GE LUNAR Corp., Madison, WI). This system uses software that has been reported to require additional modification to improve the accuracy fat content quantification [5], which was included in the current analysis. DXA and MR scans were conducted within a time span of 3 wks. To minimize potential differences between the DXA and MRI comparisons, we confirmed that body weights at the time of both examinations did not differ by more than 5% in animals used for the comparison.

2.8. Statistical analysis

All average values were expressed $\pm$ one standard deviation. The quality of automatic segmenting procedures was determined with reference to the values obtained by averaging the results obtained manually from three independent experienced observers. Thus, for each animal, the data obtained by automatic segmentation were plotted against the value obtained manually. With such a representation, the data points of an ideal automated procedure should fall on a straight line with an intercept of zero and a slope of 1 (identity). If the automated method tends to overestimate the volumes, the slope will be higher than one. Conversely, slopes lower than 1 would indicate that the tested automated method underestimates the volumes. Therefore, these slopes were considered as an “identity coefficient” which we used to quantify the accuracy of the various automated methods that were tested and to determine their tendency to overestimate or underestimate the manually determined fat contents. When required, segmented images were considered as voxel sets A and B , and compared using the Jaccard similarity coefficient, JSC , defined as

$$JSC = \frac{|A \cap B|}{|A \cup B|} \quad (4)$$

This coefficient varies from zero to one (perfect match between datasets) and is always more stringent than the Dice coefficient [6]. Coefficients higher than 0.8 were considered satisfactory, in loose agreement and on the stringent side of the threshold values adopted by Johnson and colleagues [7].

In addition, further coefficient analyses to determine overall reliability was evaluated by calculating the intraclass correlation coefficient (ICC) between the two comparative datasets for comparing between manual and automated segmentation, or comparing between DXA

body fat and MRI body fat assessments. Bland-Altman plots were also constructed for DXA vs. MRI comparisons, and percent of bias, as well as the coefficient of variability were determined ($CV = 1.96 \times \text{standard deviation}$). These statistical tests were done using MedCalc 11.3 (MedCalc Software, Ostend, Belgium).

3. Results

3.1. Water suppression

The spectral selectivity of the saturation scheme was assessed first by examining the regular and water-suppressed images (Figure 1). Application of a spectrally selective saturation pulse resulted in an efficient suppression of the signal originating from high water content tissues, such as brain and muscle (Figure 1, panes A and B). The water signal from the phantom was also significantly reduced (Figure 1B) in the water-suppressed images. The spatial homogeneity of the selective water suppression was consistent over the length of the water or oiled-filled phantom tubes, which spanned the length of the imaging voxel. The 72 mm bore radio-frequency (RF) quadrature coil used in this study was chosen to be much larger than the imaging objects. This experimental choice yielded a decreased signal to noise ratio due to imperfect coil loading. However, it was still adopted in order to improve signal homogeneity over the imaged area and suppress edge effects, which would be indicated by distorted round edges on either end of the horizontal images of the mice. Hence, the mild decrease in signal to noise ratio incurred by the use of the 72 mm resonator also resulted in very homogeneous images where the intensity of AT was remarkably similar along the length of the coil (vertical dimensions of the images) and along its axial dimension (horizontal dimensions of the images). Consequently, no significant bias field was observed in the images. The water-suppressed images were therefore used for manual segmenting of thoracic, peritoneal and subcutaneous fat (Figure 1.C.).

3.2. Manual segmentation

Three individual observers determined AT volumes on three animals in each of the lean and obese animal groups, and their results were averaged together. AT volumes obtained manually, expressed as percent of total body volume, were $18.5 \pm 4.1\%$ in the group of lean animals and $49.2 \pm 3.6\%$ in the group of obese animals. The values were in excellent agreement between observers. No significant differences were found between any of the observers for a given animal from either the lean or the obese group ($p > 0.05$). The correlation coefficients between the AT measures performed by different observers for a given animal were greater than 95% ($P < 0.01$).

3.3. Gaussian fit

A common strategy for segmenting pixel intensities is to analyze images in terms of pixel intensity bins. Briefly, the pixel intensity range of an image is divided into bins, and the number of pixels in each bin is determined and plotted as a function of the bin number, resulting in a histogram. In this alternate way of displaying the image data, the image contrast is translated into the occurrence of various peaks centered on the intensity values that best characterize certain types of tissues. Regular whole body T1-weighted images display three peaks corresponding to the AT (high intensity), the water-containing tissue (medium intensity) and low or no-water medium (bone, lungs, intestine and background air; low intensity). Sources of variation such as natural tissue composition variability or imperfect instrumentation are responsible for the spread of intensities around these average values. As a result, the choice of a threshold value can be guided by examination of the pixel intensity histogram. Here, the pixel intensities of water-suppressed images were displayed as histograms with 1000 intensity bins (Figure 2, thin blue trace). A sharp peak was observed in bins 400–700 on most of the slices in both animal groups. These peaks matched to a close

approximation of a Gaussian distribution, and their analysis with numerical fit procedures yielded accurate determinations of their mean, standard deviation and scale factors. Histograms were generated for each slice and the parameters of the Gaussian fits were determined and stored for further analysis. This procedure lasted less than 10s per dataset, compared to over 4 hrs per animal per operator for the manual segmenting. The quality of the fit was assessed by plotting the absolute difference plot (Figure 2, red trace) between the histogram and its Gaussian fit. Generally, the high pixel intensity component of the AT peak matched the fit the best (Fig. 2).

3.4. Automatic segmentation, method 1

The quality of the per-image slice Gaussian fit automatic segmentation procedure was assessed by comparing their results to those obtained by averaging the values obtained by three experimented observers. This approach proved somewhat successful, but was not very robust between animal groups. For obese animals, the best match was obtained by segmenting at the $n=3$ level ($54.5 \pm 0.3\%$ of total body volume, with an identity coefficient of 1.11), but this level yielded a strong and systematic overestimation of the AT volume in the group of lean animals ($43.9 \pm 4.3\%$ of total body volume, yielding an identity coefficient of 2.34). Conversely, segmenting at the $n=1$ level yielded the best AT volume estimates for the lean group ($15.9 \pm 1.9\%$ of total body volume, identity coefficient of 0.84), but yielded a strong and systematic underestimation of the AT volumes in the group of obese animals ($32.9 \pm 2.0\%$ of total body volume, identity coefficient of 0.67). The $n=2$ segmenting yielded intermediate results, which were not optimal but acceptable for both animal groups ($27.3 \pm 3.7\%$ for the lean group, identity coefficient of 1.46, and $43.41 \pm 1.43\%$ for the obese group, identity coefficient of 0.88).

3.5. Automatic segmentation, method 2

Similarly, the quality of the global Gaussian fit segmentation procedure was assessed against the values obtained manually as a reference. In this method, all means and standard deviations obtained from each slice of each animal regardless of group were averaged into a single value. With our experimental conditions, this resulted in an average mean intensity of 13914 ± 1321 , and a standard deviation of 2587 ± 139 (both expressed in arbitrary units). These values were used to threshold the data at the three different levels of $n=1, 2$ or 3 (Figure 3). Unlike the first method, the global thresholding yielded the same optimal threshold level ($n=2$) in both the lean and obese groups. This level slightly overestimated the volumes in the lean group ($22.54 \pm 0.86\%$, identity coefficient of 1.18), and was remarkably accurate in predicting the volumes in the obese group ($49.52 \pm 0.63\%$, identity coefficient of 0.97). Overall, the identity coefficient considering both groups was 1.00, with a confidence interval of 95% within 0.92 and 1.07. These volume predictions were more accurate than any of the predictions carried out with the first method, including those obtained with the optimal levels for each group. As a consequence, the global Gaussian fit and $n=2$ threshold level were used for the analysis of the AT in the various compartments.

3.6. Compartmentalized AT analysis

The global threshold used for total fat volume quantification was applied to the quantification of various fat stores. The average volumes determined for subcutaneous, peritoneal or thoracic AT depots were remarkably similar between the manual outlining method (lean group: $11.1 \pm 2.6\%$, $7.0 \pm 0.7\%$, $0.4 \pm 0.3\%$, respectively; obese group: $30.4 \pm 1.7\%$, $16.6 \pm 0.4\%$, $2.2 \pm 0.4\%$, respectively) and the automated method (lean group: $10.8 \pm 0.6\%$, $8.3 \pm 2.4\%$, $0.5 \pm 0.3\%$, respectively; obese group: $28.8 \pm 0.9\%$, $18.2 \pm 1.0\%$, $2.0 \pm 0.5\%$, respectively; Figure 4A–D). All compartment volumes were statistically different between the lean and obese groups, regardless of the particular quantification method. The compartment with the smallest volume was the thoracic fat. Comparisons of the segmenting

methods were also carried out in terms of the correlation at the level of each individually measured animal (Figure 5). In terms of identity coefficients, the automated segmenting tended to underestimate the subcutaneous and thoracic fat compartments (identity coefficients of 0.94 and 0.9, respectively), and to slightly overestimate the peritoneal fat store (coefficient of 1.11). The total fat composition was remarkably close to the manual outlining in terms of identity coefficient as well (coefficient 1.00). In terms of intraclass correlation coefficients (ICC), the automated segmenting tended to underestimate the subcutaneous and thoracic fat compartments (ICC values of 0.957 and 0.953, respectively), and to slightly overestimate the peritoneal fat store (ICC value of 0.945). For overall reliability, the total fat composition was also close to the manual outlining in terms of overall reliability (ICC of 0.971). Table 1 summarizes the correlation analyses regarding the intraclass correlation coefficient (ICC), percent of bias (Bias) and coefficient of variability, when comparing manual and automated segmentation approaches. Finally, the effect of the diet was observed by expressing the results in terms of the ratio of compartment or total volumes between the obese and the lean groups. In diet-induced obese mice, AT volumes were more than twice as high in the obese group than in the lean control group regardless of the quantification method or the specific AT deposit considered. The thoracic AT depot showed the greatest fold-increase with high-fat feeding (6.1 ± 3.2 according to the manual outlining and 4.8 ± 2.4 according to the thresholding approach).

3.7. Comparison of AT Automatic Segmentation to DXA Analysis

To further verify the automated segmentation procedure for measuring AT volume, a set of 32 lean (N=14) and high-fat fed (N=16) mice underwent both MRI and DXA procedures for quantifying body fat. MRI- and DXA-based approaches were highly positively correlated (Figure 6; $R^2=0.94$, $p<0.0001$). As indicated by the positive slope and y-intercept values of the linear regression, the MRI-based approach estimated higher body fat values compared to DXA (approximately 40–100% greater). Further correlative analyses indicated that the ICC was 0.938 when comparing DXA and MRI methods. The bias and CV between the two methods were 6.5 g and 5.5 g, respectively, i.e. the MRI measurements were 6.5 ± 5.5 g (equivalent to $51 \pm 43\%$ of the average body fat weight) greater than the DXA measurements. Bland-Altman plots for differences and averages in body fat between the two methods expressed as weights (g) (A) or % mean body fat weight (B) are shown in Fig. 7.

4. Discussion

Accurate and efficient methods to quantify AT from MR images are needed to facilitate studies that compare changes in body AT to changes in metabolic status or disease pathology. MR imaging is an effective approach to quantify AT in rodent models, particularly because of the ability to define anatomically-based ROI for measuring site-specific changes in AT volume. In the present study, we tested the efficacy of a Gaussian fit approach to threshold AT voxels for accurate and efficient calculation of AT volume using software algorithms that can be applied in commonly used data analysis packages such as Mathematica or Matlab. We found that a global Gaussian fit approach of water-suppressed MR images collected at 7T provides a single optimal threshold coefficient that can be used to efficiently and accurately quantify AT in lean and obese mice. This is the first report of a Gaussian threshold-based method to automatically segment water-suppressed MR images for comparing whole-body and site-specific AT volumes in lean and obese mice.

Current approaches to segment AT in water-suppressed MR images involve either manual thresholding [8,9] or a fuzzy c-means algorithm followed by a simple signal intensity thresholding approach [9]. Others have used either linear or nonlinear filters to decrease noise based on T_1 -weighted images, with linear filters predominantly including average filters, and the median filter being the most commonly used nonlinear filter for AT

segmentation [10]. Gaussian filters are commonly used to reduce noise in gray-scale images by reducing background signal that contributes to a normal distribution fit. Alternatively, in this study we applied Gaussian fitting on image histograms.

In water-suppressed MR images, we showed that a Gaussian fitting applied to the high-intensity/low pixel count signal peak of the bimodal distribution of adipose and lean tissue gray-scale pixel intensity values provides an accurate estimate of AT pixel intensities and facilitated the subtraction of background and lean-tissue pixels for establishing an AT threshold. Furthermore, we found that by applying the Gaussian fit to an average pixel intensity histogram obtained for all slices and animals (i.e., 'global thresholding'), a single Gaussian fit coefficient could be applied to all image slices and for all animals to automatically threshold AT volumes. This approach was superior to a 'per slice' Gaussian fit approach, which produced different optimal thresholds for the lean and obese groups.

Another group also used adipose segmentation in lean and obese mice with a 7T small animal MRI from parametric images based on a multi-echo sequence by measuring T_2 relaxation times of adipose tissue [10]. In their study, a combination method (combining both first echo image and T_2 parametric images) was used to perform the adipose segmentation to measure total AT volume, but not different AT tissue compartments (e.g. peritoneal and subcutaneous), which requires anatomical specification [10]. In humans, visceral and subcutaneous AT measurements have been obtained from a 3T MRI and compared to computed tomography (CT), which is considered the gold standard imaging modality for measuring visceral AT area [8]. The main disadvantage of CT, however, is exposure to ionizing radiation. In the MRI vs. CT comparison study, both methods were found to be in good agreement for measuring both visceral and subcutaneous AT, with a slight negative bias in percentage of visceral AT, perhaps due to partial volume effects at interfaces between fat and muscle or intestinal wall [8]. In their study, only T_1 -weighted MR images were used, rather than the combined water-suppressed and non-suppressed images. Recently a 3D fat-water fully-automated segmentation MR (FWMRI) imaging method at 3T MRI in humans was compared to DXA, indicating agreement between FWMRI and DXA for gross body AT and total trunk AT, and a negative bias for gross body- and total trunk lean soft tissue, which was thought to be attributed to overestimation of lean soft tissue by DXA [11]. The concordance correlation coefficient between FWMRI and DXA was 0.978. In the current study, we also observed a strong positive correlation ($R^2=0.94$; ICC = 0.938) between MR-derived AT mass and DXA-derived body fat estimates. The slope and Y-intercept of the MR versus DXA comparison of body AT mass was greater than one, indicating greater AT estimates using MR. This difference may be attributed to innate differences between the methods as MR estimates AT volume and DXA estimates fat content [2]. There are some limitations in using DXA for validating fat mass as it is known that each PIXImus DXA system gives different values for the same animals, even with correction equations and the same software [5]. The MRI and DXA comparisons are not intended to validate the MRI method, but rather to provide a comparison between MRI AT mass estimates and DXA analysis methods, as this comparison is not presently available in the literature.

An advantage of MR-based analyses of AT volume over DXA is that MR data are obtained on a per slice basis that makes regional tissue segmentation possible. By segmenting AT into subcutaneous, peritoneal, and thoracic compartments, we compared overall changes in body AT volume with high-fat feeding to compartment-specific changes. We found that 20 wks of high-fat feeding increased total AT volume by 252%, which was similar to the increases observed for subcutaneous and peritoneal compartments (268% and 231%, respectively). Thoracic AT, however, was more responsive to the high-fat diet and increased 475%. Both the absolute and percent increase in thoracic AT had the most variability compared to the

other compartments. This high variability is likely due to the small absolute size of this compartment and the difficulty in manually identifying the thoracic compartment ROI in all MR slices. Thus, even with an automated AT threshold algorithm, compartment-specific AT volume estimates are still dependent on the accuracy and precision of manual ROI selection. To facilitate accurate ROI selection, slice-by-slice ROI compartment specifications were determined for non-water suppressed MR images, which provided soft-tissue anatomy information, and then copied to the water-suppressed image slices.

By combining automated AT segmentation with Gaussian fitting for data obtained from water-suppressed MRI, it was possible to determine total and regional body fat in pre-clinical models for obesity. This automated segmentation method was validated against manual segmentation and compared with DXA analysis. This is the first report using a Gaussian-based, thresholded, automated segmentation, water-suppressed method at 7T MRI, comparing obese mice from lean mice in regional (subcutaneous, peritoneal and thoracic) adipose tissue compartments.

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Abbreviations

AT	Adipose tissue
MRI	Magnetic resonance imaging
DXA	Dual-energy X-ray absorptiometry
ROI	Region of interest
T	Tesla

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Figure 1. Effect of water suppression on transverse MR image slices and manual outlining of adipose tissue compartments

A. Normal, non water-suppressed reference image. B. MR image obtained at the same location but with the magnetization prepared, spectrally selective water suppression imaging sequence. C. Manual outlining of fat deposits in subcutaneous (labels “s”), peritoneal (labels “p”) or thoracic (labels “t”) compartments. The control tubes visible on either sides of the animal contained water (label “w”) or olive oil (label “o”). In this example, a total of 29 separate ROIs were manually outlined to account for all fat areas within the 3 designated fat compartments.

Figure 2. Gaussian fitting method used for the automated segmentation method

A Gaussian fit of a pixel intensity histogram from a central transverse slice of a water-suppressed image is presented. The Gaussian fit of the high intensity region of the pixel intensity histogram is an estimate of the AT pixels and is overlaid as a thick blue trace. The difference plot, representing the absolute value of the difference between the histogram and the fit, is presented in red and represents an estimate of the non-AT background intensity pixels. Thresholding levels for $n=1, 2$ and 3 multiples of the standard deviation for the Gaussian fit are shown. A thresholding level of $n=1$ includes few estimated background pixels but also excludes potential AT pixels. In contrast, a thresholding level of 3 would be expected to include a greater number of non-AT background pixels. Generally, the intermediate thresholding level $n=2$ provided the best comparison to the manually selected AT volume.

Figure 3. Automated segmentation of an image using the global Gaussian fit algorithm (method 2)

The starting water-suppressed image is presented in pane A. Images obtained from the automatic segmentation procedure are shown in B–D using three different thresholding coefficients (B: $n=1$, C: $n=2$, D: $n=3$). For this representative image slice, the automated segmentation yielded whole-body fat volume estimates that ranged from 2091 mm³ (B), to 1701 mm³ (C), and 1430 mm³ (D).

Figure 4. Comparison of manual versus automated compartment-specific AT volumes in lean and diet-induced obese mice

Three experienced individuals who were blinded to the animal identity manually segmented the AT volume for three different compartments: subcutaneous (A), peritoneal cavity (B), and thoracic cavity (C). These three compartments were then summed to provide the total AT volume (D). Manually segmented volumes were then compared to the automated segmentation method obtained from the global Gaussian fit algorithm. Automated volume estimates were not significantly different from the manual volume measurements for any compartment or either diet group ($p > 0.05$). Percent body fat (by volume) for total AT (D) and individual regions (A–C) were found to be significantly higher in high fat diet compared to low fat diet mice ($p < 0.05$). $N = 3$ per diet group. Values are mean $\pm$ SD.

Figure 5. Correlation analysis of manual versus automated compartment-specific AT volumes in lean and diet-induced obese mice

Three experienced individuals who were blinded to animal identities conducted both manual and automated segmentation analyses for three AT compartments: subcutaneous (A), peritoneal (B), and thoracic (C). The three compartments were summed to calculate the total AT volume (D). For each dataset, manual and automated values are represented as mean $\pm$ SD to represent the user variability. The best linear fit (blue trace) and the identity line (black trace) are shown for each compartment. The Pearson correlation coefficients were 0.93, 0.99, 0.88 and 0.98 for subcutaneous, peritoneal, thoracic and total compartments, respectively. The best-fit line slopes were 0.94, 1.11, 0.90 and 1.00 for the subcutaneous, peritoneal, thoracic and total compartments, respectively ($p < 0.05$).

Figure 6. Comparison of body fat mass measured by DXA and automated segmentation of MR images

Automated MRI-based calculation of body fat content is highly correlated to DXA-based measurements of body fat ($R^2=0.94$, $***p<0.0001$). MRI and DXA measurements were made within three weeks of each other in mice fed control or high-fat diets (total $N=32$; $N=14$ lean mice, and $N=16$ obese mice). Only mice that maintained body mass within 5% at each timepoint were used for the analysis. AT volume measurements made using the automated MRI-based algorithm were converted to fat mass using a fat density value of 0.9007 g/mm^3 . The increased fat mass measurements for MRI versus DXA-based approaches are expected because MRI-based AT volume includes non-fat components (2).

Figure 7. Bland-Altman plots comparing DXA and MRI body fat methods

Plots depict differences and averages in body fat (A) weights (g) and (B) % mean body fat weights. The blue line is the bias line, and the dotted brown lines are the 95% limits of agreement.

Table 1

Correlation comparisons in adipose tissue (AT) compartments between manual (reference method) and automated segmentation methods.

AT compartment	ICC	Bias		CV	
		Weight (g)	% Avg. AT weight	Weight (g)	% Avg. AT weight
Subcutaneous	0.957	1.7	8.3	6.0	29.3
Peritoneal	0.945	-2.5	-19.1	3.2	24.3
Thoracic	0.953	0.27	19.2	1.1	78.1
Total	0.971	-1.21	-3.5	7.5	21.4

Abbreviations: ICC = intraclass correlation coefficient, Bias = percent bias, CV = coefficient of variability.