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Technical Evaluation for *In Vivo* Abdominal Fat and IMCL Quantification Using MRI and MRSI at 3T

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Abstract

Objectives—To develop protocols that measure abdominal fat by MRI and calf muscle lipids by MR spectroscopy (MRS) at 3 Tesla, and to examine the correlation between these parameters and insulin sensitivity.

Materials and Methods—Ten non-diabetic subjects, five insulin-sensitive (IS) and five insulin-resistant (IR), were scanned at 3T. Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) were segmented semi-automatically from abdominal imaging. Intramyocellular lipids (IMCL) in calf muscles were quantified from single voxel MRS in both soleus and tibialis anterior muscles, and from MR spectroscopic imaging (MRSI), respectively.

Results—The average coefficient of variation (CV) of VAT/(VAT+SAT) was 5.2%. The inter-operator CV was 1.1% and 5.3% for SAT and VAT estimates, respectively. The CV of IMCL was 13.7% in soleus, 11.9% in tibialis anterior, and 2.9% with MRSI. IMCL based on MRSI ($3.8\% \pm 1.2\%$) was significantly inversely correlated with glucose disposal rate measured by a hyperinsulinemic euglycemic clamp. VAT volume correlated significantly with IMCL. IMCL based on MRSI for IR subjects was significantly greater than that for IS subjects ($4.5\% \pm 0.9\%$ vs. $2.8\% \pm 0.5\%$, $P = 0.02$).

Conclusion—MRI/MRS techniques provide robust, non-invasive measurement for abdominal fat and muscle IMCL that are correlated with insulin action in humans.

Keywords

magnetic resonance imaging (MRI); magnetic resonance spectroscopy (MRS); abdominal fat; intramyocellular lipids (IMCL); insulin sensitivity

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INTRODUCTION

Insulin resistance (IR) is an important predisposing factor for human type 2 diabetes and premature cardiovascular disease (1). Recent evidence indicates that lipid distribution plays an important role in the pathogenesis of IR (2,3). Consequently, there is a growing interest in evaluating the association of abdominal fat and intramyocellular lipid (IMCL) with IR.

Lipids are stored in the form of triglycerides in either adipose tissue or in lipid droplets in the cytoplasm of non-adipose cells. In skeletal muscle tissue, extramyocellular lipids (EMCL) are found within adipose cells adjacent to muscle fibers. Muscle fibers contain a separate lipid pool, IMCL, which is usually located close to muscle mitochondria and therefore serves as an important energy supply of free fatty acids for oxidation. An inverse correlation was found between the IMCL in muscle tissues and insulin sensitivity in sedentary and diabetic subjects (4,5). Conventionally, muscle lipid content has been assessed using tissue biopsies followed by either biochemical assay (6,7) or electron microscopy and morphometry (8,9). These techniques, however, are invasive and subject to sample errors, and are not suitable for use in studies requiring serial measurements (10).

Recently, magnetic resonance spectroscopy (MRS) techniques have been developed that provide a non-invasive method for distinguishing IMCL from EMCL (11,12). The separation of IMCL from EMCL is based on the different geometrical arrangement of these two sets of lipids within the highly ordered muscle tissues, leading to different resonance frequencies of the protons of these two lipid chains. EMCL are located along the muscle fibre bundles. Therefore the chemical shift of the EMCL resonance is orientation-dependent. In contrast, IMCL are located within spherical droplets with no spatial dependency of their chemical shift on the main magnetic field strength. The frequency difference between these two resonances is approximately 0.25 ppm when the lipid layer is parallel to the external magnetic field B_0 (12).

Howald et al. have proven the validity of the non-invasive, MRS determination of IMCL in a study comparing IMCL derived by electron microscopy, biochemical assays and proton MRS (10). Estimates of IMCL derived from *in vivo* MRS correlate negatively with insulin sensitivity in both humans (13,14) and animals (15). Most previous studies have employed single voxel MRS (SVS) techniques to determine IMCL. However, it is not entirely clear whether IMCL measurement is more reliable in the primarily slow-twitch soleus muscle (SO) or the relatively fast-twitch tibialis anterior (TA). Moreover, reports of 2D or 3D MR spectroscopic imaging (MRSI) techniques to evaluate muscle lipids and their distribution in different muscle groups have generally come from studies employing 1.5 Tesla (T) (16,17). With recent developments in high field MR, such as the availability of clinical systems with a field strength of 3T, it is interesting to investigate the feasibility of using MRS or MRSI to evaluate IMCL at 3T.

Since metabolic risks associated with obesity are more closely related to a central (abdominal) rather than a peripheral (gluteo-femoral) fat pattern (18), it is also relevant to measure the abdominal fat tissues. Measuring the quantity and distribution of abdominal fat in humans is generally difficult and imprecise using conventional methods such as anthropometry, ultrasound and dual-energy-X-ray absorptiometry (DEXA). Computed tomography (CT) provides a much more accurate assessment of abdominal fat distribution, but it exposes subjects to radiation and thus limits the number of repeated measurements that can be performed in a research setting (19,20). MRI may provide an accurate and safe alternative method to quantify abdominal fat, in particular, visceral fat (19,21,22). Previous studies have employed standard or water-suppressed T1-weighted images with 1.5 T scanners. To our knowledge, no studies have yet examined the feasibility and reproducibility of 3T MRI to evaluate abdominal fat *in vivo*.

The objectives of this study were: 1) to develop a robust acquisition and post-processing protocol that measures abdominal fat by MRI, and IMCL in calf muscles by SVS and MRSI methods; 2) to evaluate the reproducibility of these techniques at 3T for *in vivo* studies; 3) to examine the correlation between these parameters and estimates of insulin sensitivity in both insulin-sensitive and insulin-resistant subjects.

MATERIALS AND METHODS

Subjects

Initial studies were performed in four healthy volunteers in order to evaluate the reproducibility of the MR techniques, including quantification of abdominal fat using MRI, and calf muscle IMCL employing MR spectroscopy. To study the relationship between lipid levels and insulin action, ten sedentary, non-diabetic and non-obese subjects (eight females, two males) were enrolled in the full protocol consisting of clinical procedures and MR imaging as described below. These subjects were recruited from an ongoing study of the determinants of insulin resistance in the non-obese population. Five subjects were tentatively identified as insulin resistant and five subjects as insulin sensitive based on calculation of insulin sensitivity index (ISI) values from oral glucose tolerance test (OGTT) (23). Subsequent glucose clamp procedures confirmed that subjects in each group were insulin resistant or sensitive based on insulin-mediated glucose disposal values. All subjects displayed normal glucose tolerance as determined by oral glucose tolerance test screening. The mean age of these subjects was 34 years with a range of 23 – 50 years and the mean BMI was 24.0 kg/m² with a range of 18.8 – 27.0 kg/m². All of the subjects provided informed consent in accordance with the rules by the Committee on Human Research at our institution.

Clinical Procedures

Oral glucose tolerance test (OGTT) and insulin sensitivity index (ISI)—OGTT was employed to rule out diabetes and impaired glucose tolerance as well as to identify potentially insulin sensitive or resistant subjects. In the morning following an overnight fast, subjects consumed a solution containing 75 g glucose, with blood samples collected at –15, 0, 30, 60, and 120 minutes for determination of glucose and insulin concentrations. Plasma glucose concentrations were measured using the glucose oxidase method, and plasma insulin concentrations were determined by ELISA (Linco, St. Charles, MO). Plasma glucose levels were compared to WHO standards for diabetes mellitus and impaired glucose tolerance. Tentative assignment to insulin sensitive (IS) or insulin resistant (IR) groups was made by calculating the ISI from glucose and insulin responses to the OGTT as below (23):

$$ISI = \frac{10,000}{\sqrt{(\text{fasting glucose} \times \text{fasting insulin}) \times (\text{mean glucose} \times \text{mean insulin})}} \quad (1)$$

ISI values > 13.5 or < 7.5 were taken as indicative of insulin sensitivity or resistance, respectively, as these values represented cutoffs for the upper and lower quartiles in our population. Insulin sensitivity was quantified by hyperinsulinemic, euglycemic clamp as described prior to assignment to IS or IR groups.

Determination of body composition and regional fat distribution—Lean body mass, fat mass, and percent body fat were assessed by a whole body Dual Energy X-Ray Absorptiometry system (DEXA, Lunar Prodigytm).

Euglycemic hyperinsulinemic clamp—Assignment to IR or IS groups was based on quantification of insulin-mediated glucose disposal by a hyperinsulinemic-euglycemic glucose

clamp (24). Briefly, an intravenous catheter was placed in an antecubital vein for infusion of insulin and glucose. A second catheter was positioned in a retrograde manner in a dorsal hand vein for blood sampling. This hand was kept in a warming box thermostatically controlled at 50° C. A primed-continuous infusion of insulin (Velosulin, Nordisk, Bethesda, MD) was administered at a rate of 80 mU/m²·min body surface area for 120 minutes. Plasma glucose concentrations were measured at 5 min intervals during the clamp and maintained at approximately basal level with a variable infusion of 20% glucose.

Average glucose infusion rates were calculated for the 30-minute period between 90–120 min of the insulin infusion. Glucose disposal (M) was taken as the mean glucose infusion rate because endogenous glucose production is assumed to be zero at this level of hyperinsulinemia. Rates of glucose disposal were adjusted for differences in steady-state plasma glucose concentrations and normalized for the amount of metabolically active tissue by expressing total glucose disposal per kg body weight (M_{BW}) and per kg lean body mass (M_{LBW}) as determined by DEXA.

MR exam

All MR data were acquired with a GE Excite 3T scanner. To evaluate the reproducibility of the MR imaging and spectroscopy techniques, four healthy volunteers were scanned twice with the protocol of abdominal imaging and MR spectroscopic imaging described below, and repositioned between scans.

Abdominal Fat Imaging—Two single-slice axial abdominal fast spin echo (FSE) images (TR/TE 333/13.10 ms, flip angle =90°, NEX = 1, bandwidth [BW] = 15.63 kHz, matrix = 256 × 192, slice thickness = 11mm, echo train length [ETL] = 4) were acquired using the GE body coil, one with normal excitation and the other with water suppression. Prior to scanning, the subjects were coached through a deep breathing exercise. Subjects were then asked to hold their breath for the duration of the scan, approximately 18 seconds. The acquisition was prescribed from the sagittal scout so that the image plane passed through the center of the vertebral disc between the L4 and L5 vertebrae.

MR spectroscopy of calf muscles—Muscle spectra and image data were acquired from calf muscles using a quadrature knee coil. Single voxel Point RESolved Spectral Selection (PRESS) techniques were used to obtain spectra from both SO and TA muscles: TR/TE=2000/37 ms, 8 average without water suppression, 256 average with water suppression, BW = 5000 Hz, voxel size = 15×15×20 mm³ = 4.5 cm³, acquisition time 4:56. The PRESS box was prescribed on the axial T1-weighted FSE images of the calf. The intermuscular fat, blood vessels and bone/bone marrow were avoided as much as possible, and additional spatial saturation bands using very selective suppression pulses (25) were used to prevent potential contaminations from outside fat tissues, as shown in Figure 1(a). Two-dimensional water-suppressed PRESS MRSI were also acquired with TR/TE=2000/37 ms, phase encoding steps=16*8*1, nominal voxel size=8*8*8mm = 0.51cc, NEX = 3, acquisition time 12 minutes. The PRESS box was positioned mainly in soleus, but also covered surrounding muscles including gastrocnemius (medial and lateral), peroneus longus and brevis, and tibialis posterior, with spatial saturation bands applied, shown in Figure 1(b). Spectra for the whole PRESS box without water suppression were acquired during prescan in order to estimate the non-suppressed water. High order shimming was applied before spectroscopy acquisition to obtain a better shimming and consequently a better separation between the IMCL and EMCL peaks. The typical linewidth of water peaks was 10 – 15 Hz.

Data Post-processing

Abdominal Fat Segmentation—The segmentation was implemented using an in-house developed software package based on IDL (Boulder, CO). Due to the inhomogeneity of the coil sensitivity, an automated intensity correction algorithm based on an edge-completed, low-pass filtered image was applied to the axial T1-weighted abdominal images (26) (Figure 2a–b). Next, an elliptical contour was drawn to encompass the entire abdomen to acquire an intensity histogram of the abdomen. A threshold was chosen at the local minimum between the low intensity first peak, representing the muscle and background pixel intensities, and the higher intensity peak, representing the fat pixel intensities (Figure 2c). Contours of the subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) were then generated automatically based on this threshold (Figure 2d). The non-fat components of the segmentation, such as the spinal cord and stray single isolated islands of fat, were taken out of the fat contours manually. The abdominal girth and fat volume (SAT, VAT, and total adipose tissue, TAT=SAT+VAT) were then calculated based on the corrected contours. The segmentation results were compared between images with normal excitation and those with water suppression.

Spectroscopic data processing—The spectral data were reconstructed, corrected for phase, frequency shift and baseline distortion, and then fitted with Voigt models using methods previously developed (27,28) to estimate levels of water (4.7 ppm), creatine (Cr2 at 3.95ppm, Cr3 at 3.05ppm), tri-methyl-ammonium (TMA, ~3.2ppm), EMCL (~1.5ppm) and IMCL (1.28 ppm) for both single voxel spectra and 2D spectral data. In addition to levels for each metabolite, the fitting parameters also include linewidth for each metabolite, frequency shift for EMCL (with an assumption that frequency difference between Cr and IMCL kept constant), and Voigt model fractional parameter r , showing the proportion of Lorentzian component vs. Gaussian component in the model, $Voigt(f) = r * Lorentzian(f) + (1-r) * Gaussian(f)$ (28). For single voxel spectra, the ratios of IMCL to unsuppressed water were measured in both SO and TA. For 2D MRSI data, voxels with poor separation of EMCL and IMCL, mainly the voxels within the bone/bone marrow and blood vessels that could not be avoided during 2D acquisition, as seen in Figure 1(b), were discarded and the average ratios of IMCL to unsuppressed water were calculated using the rest of the voxels for each patient. The unsuppressed water for 2D MRSI was measured using the water-unsuppressed spectra obtained during the prescan.

Statistical Analysis

Reproducibility was estimated using average coefficient of variation (CV, defined as standard deviation/mean) for the repeated measurements of the four healthy volunteers. Since the procedure for segmentation of the abdominal fat was semi-automatic, we also examined the inter-operator reproducibility by calculating the CV of segmentation results from two operators. The Student's t test was used to examine differences between IR and IS subjects. Pearson's correlation coefficients were calculated between fat evaluation from imaging and spectral data, and rates of glucose disposal measured by glucose clamp.

RESULTS

Reproducibility of MR imaging and spectroscopy methods

In data acquired from the two repeated tests of four test subjects the average coefficient of variation (CV) of the ratio of visceral adipose tissue (VAT) to total adipose tissue (TAT=VAT+SAT) was 19.2% with normal excitation sequence and 5.2% with water-suppressed sequence. The results suggested that water-suppressed sequence was more robust for abdominal fat quantification. Therefore, abdominal fat quantification from the water-suppressed images is presented from now on. The inter-operator CV from two operators who segmented the same scans from the four volunteers was 1.1%, 5.3% and 0.5% for SAT, VAT and girth, respectively.

Figure 3 illustrates the single voxel MRS data in SO (c) and in TA (d), and the 2D-MRSI data (e) for one representative subject, demonstrating good separation between peaks of IMCL and EMCL. The average CV of the ratio of IMCL to unsuppressed water was 11.9% in TA and 13.7% in SO, respectively, for the single voxel spectral data. With 2D MRSI data, the average CV was 2.9% for the ratio of IMCL to unsuppressed water.

Clinical Characteristics

Of the ten subjects who underwent the clinical study, five subjects (four female, one male) were identified as insulin resistant (IR) and five (four female, one male) as insulin sensitive (IS). Table 1 illustrates clinical characteristics of these IR and IS subjects, including age, BMI, and glucose disposal rates. No significant differences were found between these two groups in age ($P = 0.38$) and in BMI values ($P = 0.76$). Subjects with low ISI values proved to have impaired insulin-mediated glucose disposal as determined by the clamp. M_{BW} and M_{LBM} values were significantly lower in the resistant vs. the sensitive subjects ($P = 0.007$ for M_{BW} and $P = 0.004$ for M_{LBM}).

Difference in IMCL and abdominal fat between IS and IR subjects

IMCL/water based on 2D MRSI for IR subjects was significantly greater than that for IS subjects ($4.5\% \pm 0.9\%$ vs. $2.8\% \pm 0.6\%$, $P = 0.04$). IMCL/water measured with SV in TA showed an edge significance ($P = 0.06$) between IS and IR subjects. No significant difference in IMCL measured with SV MRS in SO was found between these two groups. With this small sample size, no significance difference was found in volumes of SAT and VAT, and VAT/TAT between IS and IR subjects. Table 2 summarized the mean and SD of IMCL and abdominal fat measurement of these two groups. Figure 4 presents abdominal images with contours of SAT and VAT for an IS (a, female, 28 years, 166 cm, 58 kg, BMI = 21 kg/m²) and an IR (b, female, 33 years, 164 cm, 67 kg, BMI = 25 kg/m²) subject, respectively.

Correlation between abdominal fat, IMCL, and glucose disposal rate

IMCL/water based on 2D MRSI ($3.8\% \pm 1.2\%$) was significantly inversely correlated with both M_{BW} ($R^2 = -0.76$, $P = 0.02$) and M_{LBM} ($R^2 = -0.77$, $P = 0.04$), Figure 5(a) and 5(b). No significant correlation was found between IMCL/water measured with SV MRS and the glucose disposal rate (M_{BW} and M_{LBM}).

The volume of VAT correlated significantly with IMCL/water measured using 2D MRSI ($R^2 = 0.74$, $P = 0.03$) and IMCL/water using SV MRS in SO ($R^2 = 0.85$, $P = 0.003$), Figure 6 (a) and 6(b). The correlation between volumes of VAT and IMCL measured by SV MRS in TA was not significant.

No significant correlation was found between volumes of abdominal fat and glucose disposal rate. The correlations between these parameters were summarized in Table 3.

DISCUSSION

There is an increasing interest of using MR techniques for evaluating body fat and muscle lipids non-invasively. In particular, with development of MR techniques at high field strength and rapidly increasing usage of clinical 3T scanner, it is important to examine the feasibility and reliability of these techniques at 3T. In this study, we have developed a robust protocol to evaluate *in vivo* abdominal fat using MR imaging and calf muscle lipids using MR spectroscopy, and have evaluated the *in vivo* reproducibility of these techniques at 3T.

Single-slice breath-hold T1 weighted images between L4–L5 were used to evaluate SAT and VAT semi-automatically. We found that images acquired with water-suppression provided

better reproducibility than those obtained with normal acquisition (average coefficient of variation 5.2% vs 19.2%). This can be explained by the fact that water-suppressed images provide mainly the fat signal, which eases the determination of the segmentation threshold from the histogram. This result is consistent with previous studies by Tintera et al (29). In our study, the threshold selection needs operator interaction since the valley between the residual water signal and fat signal can be smooth and minimum values are not always easily discernable. Due to this source of potential variability, we determined inter-operator variability and the low CV indicated that the method is highly reproducible. The single-slice breath-hold method in this study provides a technique free of motion artifact. However, this study is limited to providing only one slice instead of volume images to estimate the fat distribution, and errors may be introduced due to partial voluming effect.

Single voxel MR spectroscopy was performed in both SO and TA calf muscles in this study. Reproducibility of MRS in TA is slightly better than that in SO (average CV of 11.9% in TA and 13.7% in SO). This can probably be explained by two facts: 1) the muscle fibers in TA are oriented approximately parallel to B_0 (approximately 9°) while those in SO are approximately 45° relative to B_0 (16), and therefore the separation between EMCL and IMCL is larger in TA; 2) the total fat content, especially in the EMCL compartment, is greater in SO than that in TA (17). Thus the spectral signal is more dominated by EMCL in SO, potentially affecting the accuracy of IMCL estimation. The average CV was slightly lower than that in a previous study for MRS in TA at 1.5T using jMRUI software for spectral quantification with intraday and intervisit CVs reported as 13.4% and 14.4%, respectively (30). The fitting error was calculated

for SV spectra in SO and in TA respectively as $\gamma = \frac{\sqrt{(y_i - \hat{y}_i)^2 / n - 1}}{SD}$, where y_i is the original data, $\hat{y}_i$ the fitted values, n the number of data points, SD the standard deviation of the random noise in the spectrum from a region at the right-hand end of the spectrum known to be devoid of peaks. The mean fitting error of spectra in TA was significantly lower than that of spectra in SO (9.2 ± 4.5 vs. 20.5 ± 8.3 , $P = 0.001$). The lower CV and lower fitting error with spectra in TA suggest that IMCL measurement in TA may be more reliable than that in SO.

In this study, 2D MR spectroscopic imaging provided a better reproducibility for IMCL quantification than single voxel MRS. This is probably due to the following factors: 1) the smaller voxel size used in 2D MRSI may provide a better separation of IMCL from EMCL (31); 2) during the two scans for the reproducibility study for both SVS and 2D MRSI, in the superior-inferior direction, landmarks from the tibia bone were used to make sure the spectral box was put in the same plane. However regarding in-plane acquisition, PRESS box positioning is more repeatable for 2D MRSI than that for SVS MRS.

During 2D MRSI acquisition, spatial saturation bands with very selective suppression (10) pulses were used to suppress signal from subcutaneous fat. However, it is difficult to avoid including the bone/bone marrow, blood vessels and interstitial tissues in the PRESS box. In addition, the low resolution of MRSI causes signal bleeding from these voxels that contaminate spectra in other voxels of interest. In this study, voxels that showed dominant EMCL and poor separation between EMCL and IMCL were discarded during the post-processing for IMCL quantification. Our results indicate a high reproducibility of this method but it requires manual interactions from the operator. The technique may be improved by incorporating automatic image segmentation for bone/bone marrow, blood vessels and interstitial tissues, all of which show high intensity in the T1-weighted images. Methods with a lipid extrapolation procedure or sophisticated fitting algorithms may also help to reduce the contamination of spectra from these tissues (16).

In the present study, the average ratio of IMCL to unsuppressed water was 3.0% in SO, 0.8% in TA and 3.8% with 2D MRSI. Considering the individual variation, these results were consistent with those from previous studies (14,32). In the present study, we observed that IMCL in TA is about three times lower than that in SO, which is consistent with findings in previous studies by Vermathen et al (16) although the absolute numbers are not comparable directly since they used Cr or IMCL in SO as the reference for IMCL in TA instead of unsuppressed water.

IMCL in both SO and TA muscles tended to be higher in the insulin resistant group. However, given the small sample size in this study, this increase was statistically significant only with 2D MRSI data. This result might be due to better separation of IMCL and EMCL in 2D MRSI. 2D MRSI may also help identify different spectral patterns in different muscle groups (16, 33). Our finding of a negative correlation between IMCL and insulin sensitivity is consistent with previous reports (3,13,14,34,35). IMCL also correlated significantly with glucose uptake (expressed either as M_{BW} or M_{LBM}) in the group as a whole. Although the mechanisms by which increased IMCL correlates with insulin resistance are not fully understood, the results suggest that IMCL can be an important indicator of insulin sensitivity and that MR spectroscopy provides a valuable non-invasive method for the quantification.

In the present study, we found that the VAT volume was significantly correlated with IMCL levels in calf muscles. This correlation between visceral adiposity and IMCL was also found in previous studies in lean and obese adolescents (32), and in rat models of insulin resistance (37). Central fat accumulation, rather than excess peripheral fat, is well accepted as a predisposing factor for insulin resistance (18,36). Accumulation of visceral rather than subcutaneous fat is thought to constitute the link between glucose intolerance and IMCL stores (32). However, a causal relationship between visceral fat and IMCL has not been established (37).

In conclusion, we have presented a robust protocol to evaluate abdominal fat distribution using MR imaging, and calf muscle lipids, in particularly IMCL, using MR spectroscopy techniques. The *in vivo* reproducibility of these techniques was evaluated at 3T. MRSI provides more robust measurement of IMCL than single voxel MRS. MRI/MRS techniques can be valuable non-invasive tools for measuring abdominal fat and muscle IMCL that are correlated with insulin action in human beings. Larger cohorts of subjects will be studied using developed techniques to investigate the relationship between these MR parameters and insulin action.

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Figure 1. Prescription of MR spectroscopy in calf muscles. **a:** Single voxel MRS in soleus (yellow) and in tibialis anterior (green) respectively; **b:** 2D MR spectroscopic imaging in calf muscles. Spatial saturation bands are shown as the yellow bands to suppress signals from subcutaneous fat, blood vessels and bone/bone marrows as much as possible.

Figure 2.

Semi-automatic segmentation for abdominal fat tissues using a single-slice T1-weighted image between L4–L5. **a:** The original image with a T1-weighted fast spin-echo (FSE) water suppressed sequence between L4–L5; **b:** The image after coil correction; **c:** The histogram of the image in **b**. The threshold was selected as the minimum value between the low intensity first peak, representing the muscle and background pixel intensities, and the higher intensity peak, representing the fat pixel intensities; **d:** Fat segmentation based on the threshold in **c**, with outside contour as subcutaneous adipose tissue (SAT) and inner contour as visceral adipose tissue (VAT).

Figure 3.

MR spectroscopy of calf muscles. Upper row: Single voxel MRS in soleus (b) and in tibialis anterior (c) respectively; lower row: 2D MR spectroscopic imaging. Water (4.7 ppm), creatine (Cr2 at 3.95ppm, Cr3 at 3.05ppm), tri-methyl-ammonium (TMA, ~3.2ppm), EMCL (~1.5ppm) and IMCL (1.28 ppm) were quantified from both SV and MRSI data. Spectra showed good splitting between IMCL and EMCL peaks.

Figure 4. Abdominal images for an insulin sensitive (IS) subject (a, female, 28, 166 cm, 58 kg) and an insulin resistant (IR) subject (b, female, 33, 164 cm, 67 kg) respectively. Larger volume of visceral fat was observed in the IR subject than that in the IS subject (100.57 cm^3 vs. 53.18 cm^3).

Figure 5. IMCL quantified with 2D MRSI inversely correlated with the rate of glucose disposal per kg body weight (M_{BW} , **a**) and per kg lean tissue (M_{LBM} , **b**) significantly.

a **b**

Figure 6. IMCL with single voxel in soleus (a) and with 2D MRSI (b) correlated with VAT volume significantly.

Table 1

Mean $\pm$ SD of age, BMI and glucose disposal rates (M_{BW} and M_{LBM}) for IR and IS subjects.

	Age (years)	BMI (kg/m ²)	M_{BW} (mg/kg/min)	M_{LBM} (mg/kg/min)
IR (n=5)	31.6 $\pm$ 5.3	23.7 $\pm$ 2.8	5.4 $\pm$ 1.1	8.6 $\pm$ 0.8
IS (n=5)	36.8 $\pm$ 11.1	24.2 $\pm$ 3.2	9.8 $\pm$ 2.2	14.4 $\pm$ 2.3
P	0.38	0.76	0.007	0.004

Table 2
Mean ± SD of abdominal fat and IMCL for IR and IS subjects.

	IMCL/Water (%)			Abdominal Fat (cm ³)		
	SO	TA	MRSI	SAT	VAT	VAT/TAT
IR (n=5)	3.4 ± 1.5	1.1 ± 0.4	4.5 ± 0.9	246.5 ± 97.1	86.9 ± 28.8	0.27 ± 0.06
IS (n=5)	2.6 ± 1.3	0.6 ± 0.2	2.8 ± 0.5	270.4 ± 121.2	71.4 ± 12.7	0.23 ± 0.09
P	0.38	0.06	0.02	0.76	0.32	0.47

Table 3**(a). Correlation between glucose disposal rates, IMCL and VAT**

	IMCL/water (%)			VAT (cm ³)
	SO	TA	MRSI	
M _{BW} (mg/kg/min)	R = -0.29	R = -0.37	R = -0.77	R = -0.46
	P = 0.42	P = 0.29	P = 0.03	P = 0.22
M _{LBM} (mg/kg/min)	R = -0.44	R = -0.60	R = -0.77	R = -0.45
	P = 0.24	P = 0.09	P = 0.04	P = 0.27

(b). Correlation between IMCL and VAT

	IMCL/water (%)		
	SO	TA	MRSI
VAT (cm ³)	R = 0.85, P = 0.003	R = 0.47, P = 0.20	R = 0.74, P = 0.03