Quantification of Absolute Fat Mass: A Validation Study Between Chemical-Shift MRI and Chemical Analysis

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INTRODUCTION: As the prevalence of obesity continues to rise, accurate tools for quantifying abdominal body and organ fat mass are critically needed. Fat accumulation in organs and skeletal muscles are strong biomarkers of diabetes, the metabolic syndrome, and obesity. Rapid fat quantification, particularly in organs and muscles, remains an unmet need in body composition research. Motivated by the fact the chemical analysis (CA) returns an intuitive and direct measure of absolute fat mass in animal body composition experiments, the purpose of this work was to validate an approach based on chemical-shift MRI for computing similar absolute fat mass (grams) from available proton density fat fraction (PDFF) data (%). Since MRI signals fundamentally measure proton density, and not typical SI units of volume or mass, we first describe a simple formulation that relates PDFF to absolute fat mass. Next, the approach was applied to freshly excised samples of adipose tissue, and more importantly, to mixed and heterogeneous fat and lean tissues, organs, and muscle samples from four pigs. The 97 samples were independently analyzed by gold-standard lipid extraction chemical analysis.

METHODS: ***(Theory)*** The fat-signal fraction from chemical-shift MRI reflects the ratio of protons in fat and free water in tissues. Assuming that signal confounding factors such as T_1 and T_2^* relaxation, and the multiple spectral peaks of fat [1-3] have been accounted for, the resultant fat-signal fraction is equal to the PDFF. The PDFF is not equivalent to absolute fat mass (volume) or mass (volume) fat fraction. As described previously [4,5], while the proton density and mass fractions are two fundamentally different metrics, they are nonetheless related and that for water and fat, the difference is remarkably small. The pure un-confounded proton-density signals of water and fat, S_W and S_F, for an arbitrary voxel is described by Eq. (1), where ρ is the mass density (g/ml), V is the volume of water or fat in the voxel, λ denotes the number of protons per molecule, N_A is Avogadro's number, and MW represents the molecular weight (g/mol). The numerator of the term in parenthesis has units of # of protons/mole. Dividing this by MW, the resultant unit becomes # of protons/g. Multiplication by ρ and V gives the unit of S_W and S_F as simply the # of protons. Note that the product of ρ and V is mass m. Next, we define PDFF and mass fat fraction in Eq. (2) and substitute for m in Eq. (2b) from Eq. (1). To obtain the 1.02 coefficient, we have substituted the following for water: ρ_W = 0.993 g/ml, MW_W = 18.015 g/mol, and λ_W = 2; and the following for the average triglyceride in adipose tissue: ρ_F = 0.92 g/ml, MW_F = 845.52 g/mol, and λ_W = 95.84; and dropped N_A entirely for simplicity [6-9]. Note that the expressions for PDFF (2a) and mass fat fractions (2b) are remarkably similar. We now define the approximation of absolute fat mass by PDFF within each imaging voxel v using Eq. (3), where v is voxel volume.

(1) $$S_{\{W,F\}} = \rho_{\{W,F\}} \cdot V_{\{W,F\}} \cdot \left(\frac{\lambda_{\{W,F\}} \cdot N_A}{MW_{\{W,F\}}} \right) = m_{\{W,F\}} \cdot \left(\frac{\lambda_{\{W,F\}} \cdot N_A}{MW_{\{W,F\}}} \right)$$

(2a) $$\eta_{PDFF} = \frac{S_F}{S_W + S_F}$$

(2b) $$\eta_{mass} = \frac{m_F}{m_W + m_F} \approx \frac{S_F}{1.02 \cdot S_W + S_F}$$

(3) $$m_F \approx \eta_{PDFF} \cdot (\rho_F \cdot v)$$

(Animals) Four pigs (2 males both 2 years old, 2 females ages 11 and 12 years old), with 45.5, 48.7, 78.0, 73.3 kg body weights, respectively, were used in this study. A veterinarian euthanized each animal with Telazol and Xylazine, followed by sodium pentobarbital. Organs including left and right kidneys, the spleen, the pancreas, and the heart were removed during necropsy. Additionally, samples from different lobes of the liver, the left and right longissimus muscle, subcutaneous adipose tissues from the back and sternum, and omental and peri-renal visceral adipose tissues, were obtained. Finally, random mixture samples containing both lean and fat tissues were excised. ***(MRI)*** All MRI was performed on a 3T system (Signa Excite HD 15M4, GE Healthcare) within 24 hours after necropsy. We utilized an investigational six-echo version of the IDEAL-IQ SPGR sequence. A BW of 250 kHz and 5° flip angle were used. Spatial resolution was set to 1.1 mm in-plane and 1.0 mm slices. The IDEAL software yielded separated T2*-corrected water and fat series and quantitative PDFF maps. The images were segmented using SliceOmatic (Tomovision, Inc.). Computation of fat mass via Eq. (3) was then performed and summed across all voxels of interest. We did not correct for the 1.02 coefficient. ***(Chemical Analysis)*** All samples were shipped overnight for CA by independent investigators [10] and the extracted fat masses were reported back to the MRI investigators.

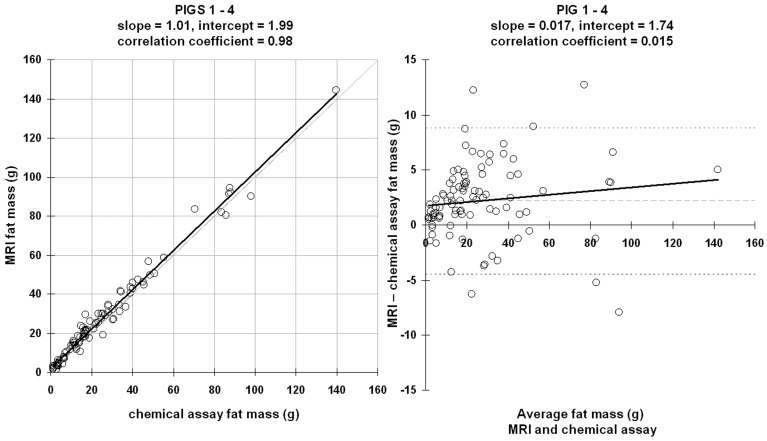

FIG. 1: (Left) Linear regression plot of fat mass between chemical analysis and MRI for all 97 samples. Light gray diagonal line in regression plot is identity. The black line is the best-fit line. (Right) Bland-Altman plot. Light gray dotted lines denote the 95% confidence intervals. The mean difference is denoted by the dashed gray line. The black line represents the best-fit line through the data.

Linear Correlation	Pig 1 (26 samples)	Pig 2 (24 samples)	Pig 3 (27 samples)	Pig 4 (20 samples)
Slope (95% CI)	1.05 (0.99, 1.11)	0.99 (0.94, 1.03)	1.07 (0.98, 1.17)	0.93 (0.85, 1.01)
Intercept (95% CI)	1.11g (-1.19g, 3.42g)	2.33g (0.63g, 4.03g)	1.52g (-0.14g, 3.19g)	2.84g (0.29g, 5.39g)
Correlation Coefficient	0.98	0.99	0.96	0.97
Bland-Altman				
Slope	0.06	-0.01	0.09	-0.06
Intercept	0.84g	2.21g	1.14g	2.60g
Correlation Coefficient	0.19	0.013	0.16	0.11

RESULTS: FIG.1 illustrates a correlation and a Bland-Altman plot of all 97 samples consolidated across the four pigs. There is excellent agreement between the two fat mass measures as the regression slope is nearly equal to one. The 95% confidence intervals for slope and intercept are (0.98, 1.04) and (1.01, 2.96), respectively. The difference between MRI and CA was 2.17±3.40g and was not statistically significant from zero. The table summarizes statistics for each pig, showing similar strong agreement between MRI and CA-derived fat mass. The differences between the two techniques were 2.66±4.36g, 1.88±2.68g, 2.73±2.50g, and 1.18±3.90g.

CONCLUSION: The ability to accurately estimate absolute fat mass with MRI from PDFF has been validated. The method allows for the computation of absolute fat mass in any arbitrary specimen, ranging from lipid-rich adipose tissue to heterogeneous organs and muscles. Future work should include a rigorous test of reproducibility and the technique's ability to non-invasively track longitudinal changes in fat mass in animal and human studies.

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