Tissue- and Magnetic Resonance-Based Metrics for Quantifying Hepatic Content: Implications for Validation Studies using Tissue as the Reference Standard

S. B. Reeder[1,2], C. D. Hines[1], C. A. McKenzie[3], and C. B. Sirlin[4]

[1]Radiology, University of Wisconsin, Madison, WI, United States, [2]Medical Physics, University of Wisconsin, Madison, WI, United States, [3]Medical Biophysics, University of Western Ontario, London, Ontario, Canada, [4]Department of Radiology, University of California, San Diego, San Diego, CA, United States

PURPOSE: Hepatic steatosis is characterized by excessive intracellular accumulation of triglycerides within hepatocytes. It is an important feature of diffuse liver disease, and is the histological hallmark of non-alcoholic fatty liver disease (NAFLD). Several quantitative MR methods, particularly single-voxel MRS and volumetric MRI methods, are being developed to detect and quantify liver disease. Validation studies are underway at many centers, comparing tissue-based measures of fat content (histology and/or triglyceride extraction) with MRS/MRI. Unfortunately, quantitative MR- and tissue-based methods ***measure inherently different (but related) quantities***, complicating direct comparison. The ***purpose*** of this work is to describe the metrics that are commonly used with histology, tissue lipid extraction, and MRS/MRI, and highlight important differences.

OUTLINE OF CONTENT: In this section we describe three tissue-based methods for quantifying fat subjectively and objectively, and highlight differences in tissue-based metrics of fat quantification with those used with quantitative MR spectroscopy and imaging methods. Differences among the tissue-based methods will be explored, as well as differences between tissue-based and MR-based measures of liver fat content.

1. Tissue-Based (Invasive)

a.i Histology-Based (Subjective): Histological evaluation has long been considered the reference standard for evaluation of liver disease, including steatosis. Subjective grading of steatosis is based on subjective assessment of ***the number of hepatocytes containing vacuoles of fat***. According to Brunt et al [1], who first described a grading/staging scheme for NAFLD, and also Kleiner et al [2], macrovesicular steatosis is graded 0–3 based on percent of hepatocytes in the biopsy involved: 0 is <5%; 1 is 5-33%; 2 is 34–66%; 3 is >66%. Reporting steatosis to the closest 5th percentile can provide additional granularity. **Figure 1** (left) shows an example of a biopsy stained with trichrome from a patient with grade 3 (80% of cells with macrovesicular steatosis). Note that ***pathological grading of biopsy does not provide a direct measurement of triglyceride concentration***, but rather the number of cells affected. Examples of different steatosis grades will be shown in the educational e-poster.

a.ii Histology-Based (Objective): Objective approaches for quantifying fat concentration from biopsy have been described [3,4]. In these approaches, digitization of slides followed by thresh-holding and automated segmentation of fat vacuoles (which appear white) can be performed. This provides an "***area-fraction" reported as a percent area of the histology preparation occupied by fat vacuoles***. Although more objective, the area-fraction, as well as subjective grading is fundamentally a 2D measurement of fat content in 3D liver parenchyma.

b. Biochemical assay (Objective) – triglyceride concentration by mass: Chemical extraction of triglycerides from liver tissue is not typically performed clinically, because extraction is destructive and the biopsy core cannot be evaluated for other histological disease features. However, ***extraction of lipids from tissue provides the best reference measure of tissue triglyceride concentration***. Triglyceride concentration is reported as the mass of the extracted triglcyeride (m_f), divided by the total mass of the specimen (m_T) (Eq 1.). The denominator of Eq. 1 can be expanded to explicitly show which components are NMR visible (m_w=mass of free water, m_f=mass of triglycerides) or NMR invisible (m_o=other components)[5].

$$\eta_{tissue} = \frac{m_f}{m_T} = \frac{m_f}{m_w + m_f + m_o} \qquad [1]$$

2. MR-based (Non-Invasive)

a. Signal fat-fraction: Multiple investigators use single-voxel MRS, and chemical shift based water-fat imaging methods to provide a quantitative measure of "***signal fat-fraction***". The signal fat-fraction is the ratio of the separated signal from fat, divided by the total signal from water and fat, which removes B_1 coil sensitivity effects. Unfortunately, numerous biological, physical, and technical "confounding" factors influence the relative MR signals from fat and water. Therefore, the ***signal fat-fraction is a confounded measure of steatosis***, may not reliably reflect the fat content within the liver, and is not a reliable or standardized biomarker of steatosis.

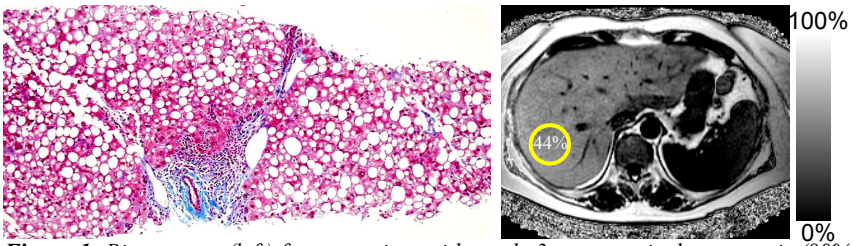

Figure 1*: Biopsy core (left) from a patient with grade 3 macrovesicular steatosis (80% of cells affected) shown on trichrome stain. Proton density fat-fraction map from the same patient demonstrates 44% proton density fat-fraction. This example explicitly shows how histology and imaging provide fundamentally different measures of liver fat.*

b. Proton density fat-fraction: Confounding factors for MRS include [6,7]: J-coupling, T1, T2, and the multiple peaks in a fat spectrum. Confounding factors for MRI methods include [8-11]: T1, T2*, spectral complexity of fat, noise bias and eddy currents. In this work, we will describe these confounding factors and how they can be addressed. When confounders are addressed, the signal fat-fraction is equivalent to the proton density fat-fraction (PDFF), i.e.: the ratio of the unconfounded fat signal (S_f) and the sum of the unconfounded water and fat signals (Eq 2). Since the relative proton densities of fat ($\rho_f\lambda_f/MW_f$) and water ($\rho_w\lambda_w/MW_w$) are almost equal [5,12] (ρ = density of water (fat), λ = number of protons per molecule of water (fat), MW = molecular weight), Eq. 2 is equivalent to Eq. 3, which is written in terms of mass instead of signal.

$$\eta_{PD} = \frac{S_f}{S_w + S_f} \qquad [2] \qquad \eta_{PD} \approx \frac{m_f}{m_w + m_f} \qquad [3]$$

Proton density fat-fraction is an inherent tissue property that reflects the tissue fat content. It is independent of platform, field strength and protocol, making it a useful biomarker of hepatic steatosis. Fig. 1 (right) shows a PDFF map from the same patient.

SUMMARY: Thorough validation of MRS/MRI methods that measure PDFF requires comparison to tissue-based reference standards such as tissue triglyceride concentration and/or histological evaluation from biopsy. It is critical for researchers and clinicians performing validation studies of non-invasive imaging methods to understand the differences between tissue metrics of fat content (e.g. steatosis grade, area-fraction or extracted triglyceride concentration) and PDFF measured from MRS/MRI.

References: 1. Brunt et al AJG 1999, 94:2468-2474
2. Kleiner et al, Hep, 2005, 41:1313-1321
3. Marsman et al Hum Pathol 2004, 35:430-5
4. Zaitoun etl al J Clin Pathol 2001, 54:461-5
5. Longo et al JMRI 1995, 5:281-5
6. Pineda et al Radiol 2009, 252:568-76
7. Hamilton et al NMR in Biomed, 2010 in press
8. Hussain et al Radiol 2005, 237:1048-55
9. Bydder et al MRI, 2008, 26:347-59
10. Yu et al, 2008 MRM, 2008, 60:1122-34
11. Yokoo et al Radiol 2009, 25(1):67-76
12. Reeder et al ISMRM 2009, pg 211