

High-throughput automated approach to quantitative evaluation of hepatic fat fraction in mice

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Introduction

MR-based techniques are well suited to the assessment of hepatic steatosis due to their ability to non-invasively differentiate chemical species [1,2]. Recently, considerable attention has shifted from spectroscopy- to imaging-based approaches [2] to benefit from the shorter scan time and the capacity to map intra-organ heterogeneity to minimize sampling errors. In the context of preclinical research in obesity and type 2 diabetes mellitus, fast, sensitive readouts are needed that can achieve a throughput on par with conventional terminal procedures. The non-invasive nature of MR techniques allows for reduced animal use and longitudinal evaluation of treatment effects. In this work, we describe a highly automated, high-throughput approach for evaluating hepatic fat fraction in mice which allows for acquisition and analysis of data from 35 mice per day.

Methods

Imaging was performed on a Bruker PharmaScan 4.7T MR scanner (Bruker Biospin, Ettlingen, Germany) with a 6-cm inner diameter birdcage coil. Calibration steps (shimming, base frequency, transmitter power, and receiver gain) were performed automatically by the system-default methods. The IDEAL method [3] with three optimized echo times [4] was selected for its robustness. Images were acquired using a modified multislice gradient echo sequence with 3 slices, 6 repetitions, navigator echo, repetition time of 200ms, echo times of ~2.79, 3.27, and 3.76 ms, 20-degree flip angle, and scan time of 5 minutes 46 seconds. Reconstruction was performed using hierarchical IDEAL [5]. The T_2^* effects were minimized by choosing the shortest optimized echo times and applying retrospective correction that aimed at minimizing the signal of the “blank” peak in the hierarchical IDEAL algorithm. The T_1 effects were minimized by using a low flip angle and a long repetition time [6]. Navigator-based retrospective motion compensation was used to reduce motion artifacts, which affected signal intensities and quantitation, allowing for fewer averages and a shorter overall acquisition time.

The acquisition process was driven by a push-button macro that interfaced with the scanner software. Reconstruction and image analysis were performed by custom program in Matlab (The MathWorks, Natick, MA, USA). The analysis software allowed interactive delineation of the region of interest (ROI) for on-the-spot analysis. The average fat fraction was calculated according to:

$$\text{Fat fraction} = \text{Real} \left(\frac{F(F+W)^*}{|F+W|^2 + \sigma^2} \right) 100\% \quad \text{where } F \text{ and } W \text{ signify the fat- and water-only signals integrated over the entire ROI, respectively. } \sigma^2 \text{ denotes the noise variance for regularization.}$$

A sustainable throughput of 13 min/mouse (35 mice/7.5 hr day) was achieved by minimizing user input with the push-button macro and rapid analysis software. Automations also allowed interleaved preparation, acquisition and analysis of multiple mice by a single operator. Intra-day test-retest studies were conducted with mice of varying sizes. Each animal was positioned and imaged separately over 6 sessions in one day. Data from a separate cohort of mice with a mean hepatic fat fraction ranging from 1.8% to 12.6% was analyzed independently by three analysts. Subsets of these studies illustrating the reproducibility of macro-driven acquisition and inter-operator variability of the Matlab-based analysis user interface are presented below.

Results

Figure 1 presents images of an axial slice through the center of the liver from hierarchical IDEAL reconstruction. Figure 2 shows the fat fraction maps and quantification from two examples of test-retest reproducibility from a 60g ob/ob mouse (top row, mean±SD = 19.7%±0.6%) and a 25g lean C57/BL6 mouse (bottom row, 3.2%±0.2%). Figure 3 illustrates the inter-operator variability with an overall standard deviation of 0.29%. Figure 4 demonstrates an application of this technique to 80 mice with diet-induced obesity, showing that heavier mice, particularly those with increased total body fat, tended to have higher hepatic fraction. Whole-body adiposity was measured by non-localized spectroscopy.

Conclusion

In this work, we combined macro-driven automation, IDEAL acquisition, retrospective motion compensation, hierarchical IDEAL reconstruction and rapid interactive image analysis to transform an MR-based measurement of hepatic fat fraction into an automated push-button process that allows a daily throughput of 35 animals including acquisition and analysis.

References [1] Hussain HK et al. *Radiology* (2005) 237 :1048-55. [2] Kim H et al. *MRM* (2008) 59:521-27. [3] Reeder SB et al. *MRM* (2005) 54 :636-44. [4] Pineda AR et al. *MRM* (2005) 54 :625-35. [5] Tsao J, Jiang Y. *ISMRM* 2008. 653. [6] Liu CY et al. *MRM* (2007) 58 :354-64.

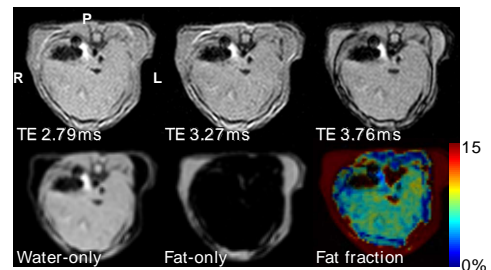


Fig. 1: Representative liver images from diet-induced obese mouse at 3 echo times (top) and from reconstruction (bottom)

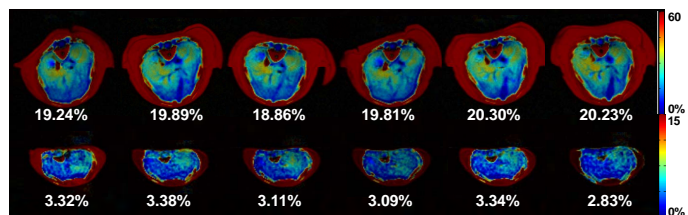


Fig. 2: Results demonstrating test-retest reproducibility over 6 runs.

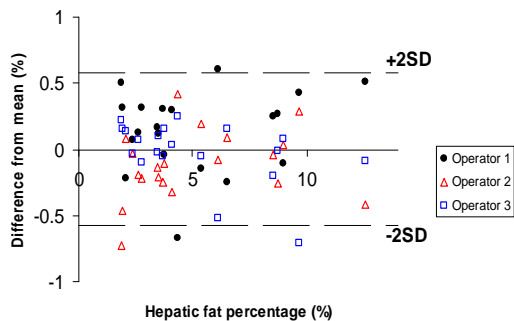


Fig. 3: Plot showing inter-operator variability in image analysis based on deviation from mean plotted against mean observed hepatic fat fraction.

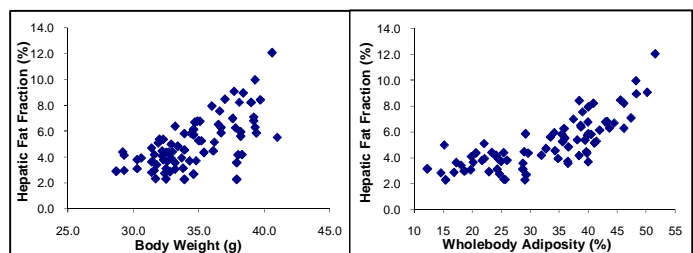


Fig. 4: Hepatic fat fraction data from semi-automated readout track well with bodyweight and whole-body adiposity in diet-induced obese mice.