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AMDCC Pilot and Feasibility Progress Report
September 2010

Novel MRI Imaging Biomarkers for Diabetic Complications

The specific aim of the funded research was **to develop MRI imaging techniques to assess the development of nephropathy and fatty liver disease in the db/db mouse.** During the funding period, significant progress has been made in developing and optimizing these techniques for both kidney and liver imaging as outlined below.

Subaim A. To Develop MRI Imaging Techniques to Assess Nephropathy

In the original application, we proposed to use Magnetization-transfer (MT) MRI to assess the presence of collagen (fibrosis). However, a number of important technical limitations with Magnetization Transfer MRI (ex. B1 spatial heterogeneity) suggested that MT-MRI would not be an optimal method for detecting renal fibrosis. As an alternative, Diffusion MRI, specifically, diffusion tensor imaging (DTI) is a quantitative imaging assessment of tissue water mobility that detects both tissue microstructural changes and tissue perfusion. We have recently identified changes in the fractional anisotropy (FA) of human diabetic patients with moderate to severe diabetic nephropathy using this technique (**Figure 1**).

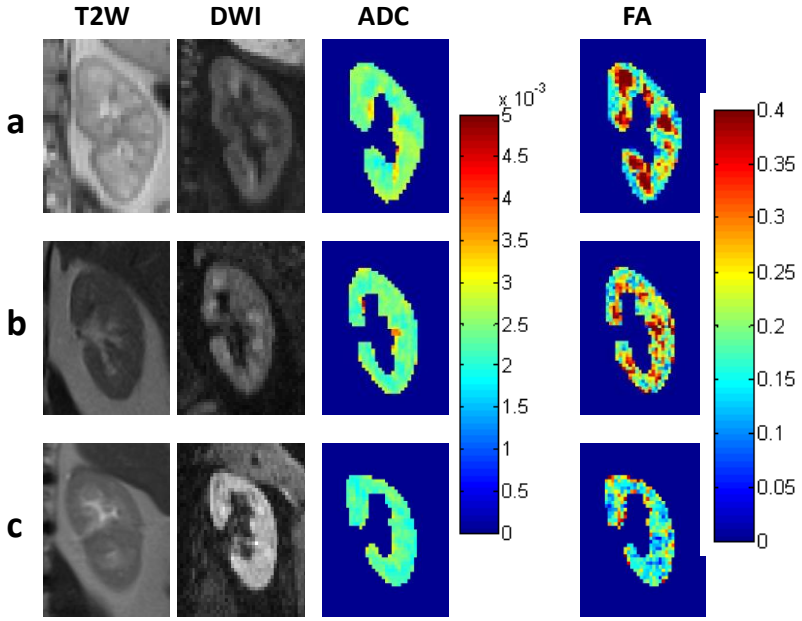


Figure 1. Representative T2-weighted images and corresponding diffusion maps for **(a)** a normal control subject (eGFR = 90), **(b)** a diabetic subject with moderate disease (eGFR = 76), and **(c)** a diabetic subject with more advanced disease (eGFR = 32). For each subject, MRI results presented include a Coronal T2-weighted images (T2W), diffusion-weighted images (DWI, b = 400 sec / mm^2), Fractional Anisotropy maps (FA), and Apparent Diffusion Coefficient maps (ADC). Note the progressive decrease in medullary FA for diabetic subjects in comparison to the normal control.

Having already optimized the technique for human/clinical scanners, we then sought to apply DTI to mouse imaging using a 7-Tesla small animal MRI scanner. With further optimization on the small animal MRI scanner, we were able to obtain Diffusion MRI data, specifically DTI-MRI data, on db/db and age-matched control mouse kidneys. Representative FA maps are shown in **Figure 2**. Note the decreased FA observed in db/db kidneys is similar to the findings in human diabetic patients (**Figure 1**). These findings suggest that the db/db mouse may be a useful model for delineating the pathophysiologic processes underlying the FA changes seen in human diabetics. Studies are currently underway in younger db/db mice to determine the time point at which these changes occur in the disease process and define the pathologic and clinical changes evident at that time.

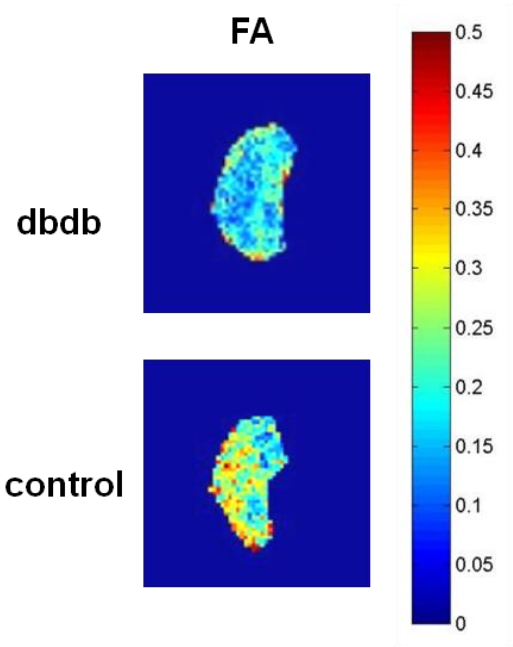


Figure 2: Diffusion MRI Fractional Anisotropy (FA) maps of kidneys from a 12-month old db/db mouse (upper panels) and lean control (lower panels). FA is decreased in the db/db kidney compared to the control.

Subaim B. To Develop MRI Imaging Techniques to Assess Fatty Liver disease.

As proposed in the original application, we have optimized IIterative Decomposition of water and fat with Echo Asymmetry and Least-squares estimation (IDEAL) for imaging fatty liver disease. For these optimization studies, we employed a fatty liver model (based on a low and fat diet) to determine the best parameters for imaging db/db mice.

The improved IDEAL MRI acquisition technique has been fully validated in lipid and oil phantoms as well as for in vivo mouse applications in vivo. We have substantially improved our MRI acquisition techniques by incorporating an asymmetric RARE (Rapid Acquisition with Relaxation Enhancement) method which utilizes multiple echoes for each acquisition to reduce our overall acquisition time by a factor of four to increase throughput. We have also begun to utilize combined ECG / respiratory gating in our liver MRI acquisitions as cardiac motion can substantially alter liver images, especially in obese animals with expanded liver volumes due to steatosis. The net result of these improvements has been successful validation of the quantification techniques for both liver fat fraction and subcutaneous and peritoneal adipose tissue volumes. A summary of these results for cohorts of mice on either high fat diet (HFD) or low fat diet / normal chow (LF) are shown in **Figure 3**. Note the relatively small error bars on all of the data sets. Tissue analysis has shown that the increased variation observed in the HFD mice is due to phenotypic variability rather than variation from the image acquisition and analysis.

We have also developed a new Diffusion-Prepared Fast Imaging with Steady-State Free Precession (DP-FISP) -MRI acquisition that enables fast and effective body diffusion MRI assessments on high field MRI scanners. We have recently utilized this technique to detect hepatic fibrosis in a PCK rat model of periportal fibrosis (**Figure 4**).

Studies are currently underway in db/db and control mice using these two techniques to quantitative hepatic fat content and assess hepatic fibrosis and correlate these imaging findings with histologic changes.

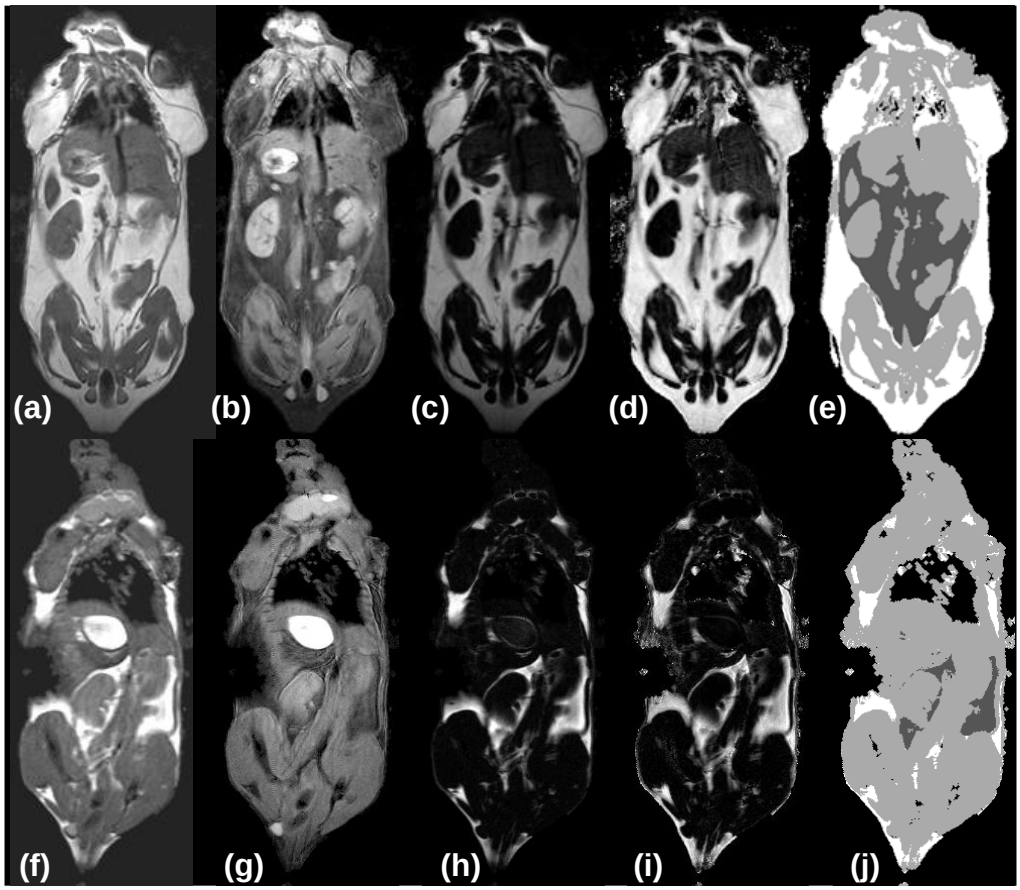
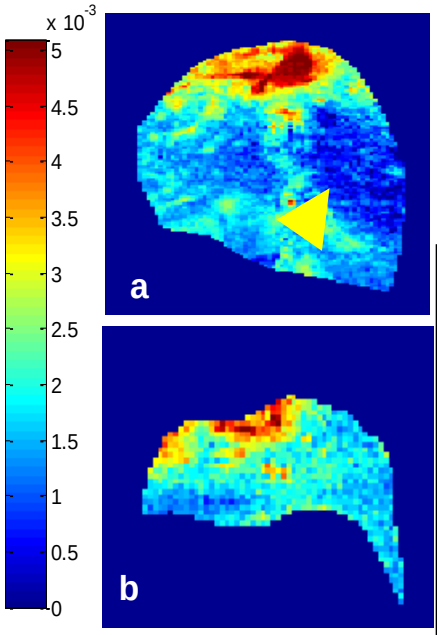


Fig 3. Raw and processed MRI images from HFD (a-e) and LFD mice (f-j) show *in vivo* measurement of adipose tissue depots. RARE images (a, f) are used to reconstruct water (b, g) and fat (c, h) images with enhanced water-fat contrast. Relaxation compensated fat fraction maps (d, i) enable semi-automatic tissue volume quantification as shown in the label images (e, j). In the label image, non-visceral adipose tissue is white, visceral adipose is dark gray, muscles and organs are light gray, and air is black.



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Mean Liver ADC

($x10^{-3}$ mm^2/sec)

4-mos SD	4-mos PCK
2.11	1.81

Figure 4: In Vivo ADC maps from 4-month-old (**a**) PCK and (**b**) SD rat livers. ADC map for PCK rat liver shows large region of the liver (left lobe, right side of image) with low ADC values (<$1.0x10^{-3}$ mm^2/sec, yellow arrow) which may be indicative of periportal fibrosis. (**c**) Mean liver ADC for the PCK is also ~15% lower than for the SD liver.