Evaluation of Nonalcoholic Liver Disease using $^{23}$Na MRI and Shift Reagent-aided $^{23}$Na and $^{31}$P MRS

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Introduction
The ability to noninvasively detect diffuse chronic liver diseases, especially nonalcoholic fatty liver disease (NAFLD), is greatly needed since currently the gold standard for diagnosis is biopsy. One possible imaging modality that takes advantage of an altered transmembrane Na$^+$ gradient in diseased tissue is $^{23}$Na MR. The current study uses single quantum (SQ) and triple quantum-filtered (TQF) $^{23}$Na MR techniques to show variations in the observed signal intensity (SI) that correlates with disease progression. To quantify these changes observed with $^{23}$Na MRI, shift reagent (SR) and $^{23}$Na and $^{31}$P MRS techniques were employed to determine the relative intra- and extracellular spaces (rICS and rECS, respectively) and intra- and extracellular Na$^+$ concentrations ([Na$^+$]i and [Na$^+$]e, respectively). To determine whether the TQF SI came from the intracellular space or whether increased extracellular matrix (ECM) proteins contributed to the signal, TQF SR experiments with $^{23}$Na and T$_2$ assessment were conducted before and after SR infusion.

Methods
Wistar rats (~250 g) were placed on methionine- and choline-deficient diet (MCDD), after baseline SQ and MQF $^{23}$Na MRI. Additional data were collected at 2, 5, and either 10 or 15 weeks after initiating MCDD treatment. MR data were acquired with a Varian 9.4 Tesla horizontal bore system. 3D SQ transaxial $^{23}$Na MRI were obtained with a home-built loop-gap resonator tuned to 106 MHz. The $^{23}$Na SI MRI were collected using a gradient-echo (GE) imaging sequence and following imaging parameters: TR = 50 ms, TE = 4.5 ms and 10 min total imaging time. TQF $^{23}$Na MRI employed the same parameters as used for SQ $^{23}$Na MRI except TR = 100 ms and 50 min total imaging time. Rats were surgically prepared for infusion of TmDOTP through the external jugular vein. A 2 cm diameter surface coil tunable to 106 MHz for $^{23}$Na and 163 MHz $^{31}$P spectra was placed over the exposed liver with proper placement being confirmed by minimal if any observable phosphocreatine peak in the $^{31}$P ATP spectra. $^{23}$Na MRS were collected with a simple one-pulse sequence. $^{23}$Na T$_1$ was measured using a pulse-burst saturation recovery pulse sequence consisting of 10 saturation pulses followed by an incremental delay (16 values ranging from 0.05 to 200 milliseconds), a 90° observed pulse and acquisition with Cyclops phase cycling. $^{23}$Na T$_2$ and T$_2$ were measured using a Hahn SE sequence consisting of a composite 180° pulse. The TE was varied from 0.06 to 40 milliseconds. The instrument dead time of 10 microseconds was included as a part of the TE. The relaxation times were computed by fitting a plot $^{23}$Na resonance area versus TR or TE to a mono-exponential function for T$_1$ and a bi-exponential function for T$_2$. The TQF $^{23}$Na MRS sequence employed a MQ preparation time (τ) and evolution time (τ) of 3 µs in order to maximize the SI [1]. Due to the dependence of the TQF signal on T$_2$ and T$_2$, a second TQF experiment was performed by varying τ from 0.06 to 40 milliseconds. Histologic samples were fixed in formalin and stained with H&E and trichrome stains.

Results
SQ and TQF $^{23}$Na MRI SI decreased to a minimum at week 5 (0.33±0.01 and 0.11±0.01, respectively) and then peaked at week 15 (0.44±0.04 and 0.18±0.01, respectively) becoming significant to baseline. These data negatively correlate with the lipid content observed with $^1$H MRS (data not shown). For the SR experiments, an increase in [Na$^+$]i was observed at week 15 (58.6±3.34) compared to baseline (28.9±8.6). No change was observed between intra- and extracellular T$_1$ measurements. T$_2$, did not vary between timepoints, however, T$_2$ did increase slightly (~50%) at week 15 compared to baseline. Although the [Na$^+$]i did increase, the TQF $^{23}$Na MRS data showed that a large component of the TQF signal arose from the extracellular space. Therefore, the increase in TQF $^{23}$Na MRI SI is due to not only increased [Na$^+$]i, but also a large increase in the macromolecules associated with fibrosis development. No variations were observed in pH, [Mg$^{2+}$], or β-ATP/Pi between timepoints. Histological data correlated with the data (i.e., increasing fibrosis with time and cirrhosis development at week 15).

Conclusion
SQ and TQF $^{23}$Na techniques are sensitive to cellular and tissue damage caused by diffuse liver diseases, prompting their further development and clinical translation. TQF $^{23}$Na MRI may be more useful in detecting the severity and progression of liver damage than SQ $^{23}$Na MRI since it depends on [Na$^+$]i and the accumulation of ECM macromolecules [2].