Separating Signals from Intra- and Extracellular Water Compartments in Rat Skeletal Muscle *In Vivo* Using MEMRI

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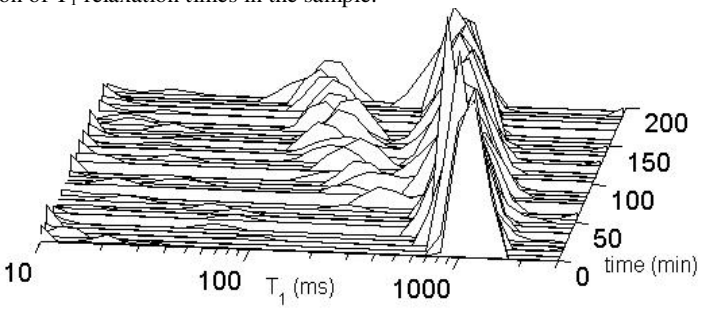
Introduction. Tissue inter-compartmental equilibrium water exchange can significantly affect the quantitative analysis of various *in vivo* MR parameters. One method for investigating equilibrium-water-exchange effects is NMR Relaxography,[1] which can employ an MR contrast agent to selectively modify the T_1 relaxation time of one tissue compartment relative to the other. In the absence of a contrast agent, the inter-compartmental water exchange rate is significantly faster than the difference in longitudinal relaxation rates ($R_1 = 1/T_1$) in each compartment. In this case, a mono-exponential T_1 relaxation time would be measured. However, selective addition of an MR contrast agent (of sufficient concentration) to one compartment can increase the T_1-relaxation-rate difference between the two compartments (relative to the inter-compartmental exchange rate; which remains constant); potentially moving the system into an intermediate- or slow-exchange regime. If the slow-exchange regime can be achieved, then the MR signal contributions from each compartment can be separated on the basis of their T_1 relaxation time differences. In the simplest case, a bi-exponential T_1 relaxation time would be measured. One impediment to achieving the slow-exchange regime *in vivo* has been the difficulty in attaining the necessary contrast-agent concentration in one of the tissue compartments. In previous *in vivo* attempts in rat skeletal muscle using Gd(DTPA), only the intermediate-exchange regime could be achieved.[2] In this study, manganese-enhanced MRI (MEMRI) has been investigated as a method for separating and identifying the MR signals from intra- and extracellular water compartment in rat skeletal muscle *in vivo*.

Materials and Methods. The experiments were performed on a GE CSI-II 2.0T/45-cm imaging spectrometer operating at a ^{1}H frequency of 85.56 MHz. A 22 mm (ID), 4-turn solenoid RF coil was placed around the right thigh of male Sprague-Dawley rats. In order to prevent the RF field from extending outside the longitudinal edges of the coil, a doughnut-shaped piece of copper foil was placed at each end. This made it possible to perform localized spectroscopy using only the RF-field profile of the coil itself. $MnCl_2$ was infused through a catheter inserted in the femoral vein. Five rats, with an average weight of 320 g, were studied at six different [Mn^{2+}] (20, 30, 40, 50, 60 and 70 mM); each infused successively over periods of 32 min at a flow rate of 0.0266mL/min. The total accumulated dose was 0.715 mmol/kg Mn^{2+} after 192 min. During infusion, T_1 was measured over 6-min intervals using a standard Inversion Recovery (IR) pulse sequence with an adiabatic inversion pulse. The data were analyzed by Inverse Laplace Transform (ILT); which, when performed on the IR data, yields the distribution of T_1 relaxation times in the sample.[1]

Results. A typical series of T_1 distributions, as a function of infused [Mn^{2+}], over time is shown in the Figure (Exp. No 5). At low [Mn^{2+}] only one peak (located around 1170 ms) is observed. This is indicative of the fast-exchange regime, where the difference in the T_1 relaxation rates (ΔR_1) between the two compartments is small relative to the inter-compartmental equilibrium water exchange rate. However, as the administered [Mn^{2+}] increases over time, the mean of the single distribution shifts to lower T_1 values. At an infused [Mn^{2+}] = 50 mM, a second distribution appears. If there is no significant transport of Mn^{2+} into the skeletal-muscle intracellular (IC) space over the experimental time course, then the fast T_1 component can be assumed to arise from water in contact with Mn^{2+} in the extracellular (EC) space (as well as in equilibrium with the vascular water). The appearance of two T_1 distributions also heralds the onset of the slow-exchange regime. The means of the T_1 distributions (and the fractional population, p1, of the short-T_1 component; p2 = 1.0 – p1) at the highest (final) infused [Mn^{2+}] are given in the Table and were similar for all experiments. The fraction of the shortest component (p1) corresponds well to values for the skeletal muscle EC fraction reported in the literature.[3]



Exp. no	T_1 initial	T_1-1 final	T_1-2 final	p1 final
1	1197	134	539	0.25
2	1175	155	616	0.24
3	1230	138	530	0.27
4	1152	130	480	0.26
5	1170	95	473	0.24
Overall (SD)	1185 (30)	130 (22)	528 (57)	0.25 (0.01)

Discussion and Conclusions. A similar study using Gd(DTPA) was not able to separate the water signals from the two compartments in rat skeletal muscle.[2] A shoulder was observed at highest [Gd(DTPA)]; perhaps indicating the approach of the slow-exchange regime. In the Gd(DTPA) study, the shortest T_1 value attained was ~600 ms at the highest dose. In our study, a value of ~500 ms was observed at the onset of peak separation, with a value of ~350 ms reached at the highest [Mn^{2+}], indicating a significantly higher [Mn^{2+}] and/or relaxivity for Mn^{2+} in the EC space. Mn^{2+} bound to macromolecules in the plasma and interstitial space can result in a 5-fold increase in relaxivity vs Mn^{2+} in water.[4] The enhanced relaxivity, in combination with [Mn^{2+}], appears to be sufficient to achieve the slow-exchange regime; allowing separation of the EC and IC water signals. By contrast, the relaxivity of Gd(DTPA) is the same in water and plasma,[3] which may explain why only the intermediate-exchange regime was attained in (2), even at plasma [Gd(DTPA)] as high as 10 mmol/L. The ability to differentiate between IC and EC water compartments *in vivo* using MEMRI would have important implications for studying inter-compartmental equilibrium water exchange in skeletal muscle under a variety of circumstances as well as other applications.

References. **1**. Labadie C, *et al*, J. Magn. Reson. Ser B, 1994;105: 99-112. **2**. Landis CS, *et al*, Magn. Reson. Med., 1999; 42: 467-478. **3**. Donahue, KM, *et al*, Magn. Reson. Med., 1995; 34: 423-432. **4**. Wendland, MF, NMR Biomed., 2004; 71: 581-594.