Metabolite T1 relaxation enhancement by spectrally-selective excitation

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Introduction. The efficiency and signal to noise ratio (SNR) of nearly all Magnetic Resonance Spectroscopy (MRS) experiments are governed by T1 relaxation of the metabolites, which determines the optimal repetition rate and hence the SNR per unit time ratio. Accurate knowledge of metabolite T1s is also paramount for metabolite quantification[1,2], and understanding spin lattice relaxation can also open valuable vistas to understand the structure and function of *in-vivo* systems. In protein solution NMR, it is well-established that the effective T1 of specific protein resonances may be modified by the use of band-selective excitation: the abundant reservoir of water- and backbone- protons, if left unperturbed, can contribute to T1 relaxation enhancement via cross-relaxation and/or exchange effects[3,4]. Such T1 modulation has to our knowledge never been sought or observed in metabolites residing in biological tissues –even if experiments observed slight changes in metabolite signals upon water inversion at lower fields[5,6]. Since the correlation time of metabolite in tissues could be expected to be longer than their free solution counterparts, we hypothesized that the water and macromolecular protons in tissue could also affect T1 relaxation rates of both upfield, non-exchanging methyl resonances of metabolites, as well as the downfield exchanging (and yet unassigned[5]) resonances. Thus, we designed a spectrally-selective Spin Echo sequence and studied the T1 relaxation properties in the excised mouse brain.

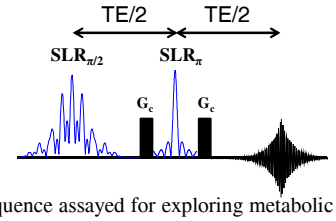

Fig. 1. SE-SLR sequence assayed for exploring metabolic relaxation enhancement, relying on multiband excitation and selective refocusing of only the selected metabolites.

Methods. All experiments were performed on excised fresh mouse brains (*N=4*) , immersed in Fuorinert within a 10mm NMR tube and scanned on a 9.4T Bruker Avance magnet with a micro5 imaging probe. Care was taken to ensure the structural integrity of the fresh specimens. A multiband $\pi/2$ 32ms excitation pulse was designed to encompass two 120Hz bands covering the upfield Lac and NAA resonances, respectively, and another band (200Hz) covering the resonances of Cre and Cho. The pulse was designed such that less than 1E-4 of the water signal is excited. A similar (single-band) pulse was designed for downfield excitation. A single-band refocusing pulse flanked by two crusher gradients was designed for spectrally-selective refocusing. All pulses were designed by the Shinnar-Le Roux (SLR) algorithm[7], and combined into a Spin-Echo SLR sequence (SE-SLR, Fig. 1). No water suppression was used for these SE-SLR experiments. Progressive Saturation (PS) experiments[1] with TR varying between 0.544s and 8.544s were then performed to obtain complete T1 relaxation curves. For comparison, T1 relaxation curves were obtained using WATERGATE[8] (WG, water-suppressed) PS spectra. For all experiments, DS=8, NA=4 and TE=144ms. The entire measurement took less than 25 minutes per method, such that tissue deterioration was not a factor. The T1 values were extracted from fitting the data to $M(TR)=M_0(1-exp(-TR/T1))$. A paired t-test was subsequently performed. Identical measurements were performed on a metabolic phantom.

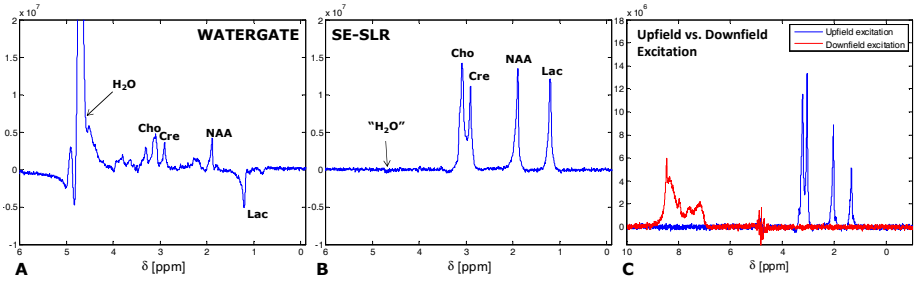

Fig. 2. **(A)** WG and **(B)** SE-SLR with no water suppression spectra. TR/TE = 1000/144ms. NA=4. **(C)** Upfield vs. Downfield SE-SLR excitation at TE=~40ms, NA=4. Note the remarkable intensity of downfield signals; WG spectra showed only noise downfield of water.

Results and Discussion. Figure 2A-B directly compares WG and SE-SLR MRS spectra. Although the water signal was not entirely suppressed in the WG spectrum, robust quantification of the metabolite T1 is feasible. On the other hand, the SE-SLR spectrum shows no water signal whatsoever; the resonances of interest are excited with no baseline distortions and the ensuing methodology yields exceptional spectral quality. Notice that the Lac signal does not evidence J-modulation, since its coupled partner is not refocused. Figure 2C shows yet another remarkable property of spectrally-selective excitation: very intense (and currently unassigned[5]) signals were observed upon excitation of the downfield region, revealing fine structure of resonances; by contrast, no downfield signal could be detected in the corresponding WG spectrum even when the number of averaged was increased by an order of magnitude (not shown).

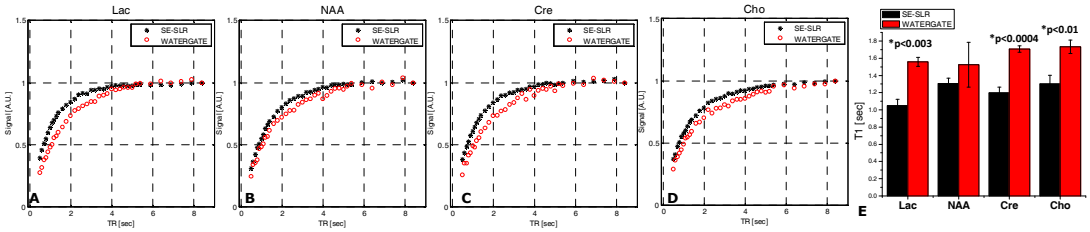

Fig. 3. Representative T1 decay curves of upfield metabolites **(A-D)** and statistical analysis **(E)**. Note the faster buildup of the metabolite signal for SE-SLR.

Figure 3A-D shows typical relaxation curves for the four upfield resonances (for display purposes, the signals were normalized to the last point for each curve). Clearly, for most resonances, the T1 relaxation curve for the SE-SLR sequence evidences a faster buildup than its WG counterpart, suggesting shorter T1s in the former. A statistically significant 30-50% change in T1 values was observed (Fig 3E) for Lac, Cre and Cho but not for NAA. Using the SE-SLR sequence, we were also able to measure the T1 of downfield resonances at 9.4T, for the first time, where we discovered very short (~0.6-0.8s) T1s for several of the downfield peaks, and a longer T1 (~1.9s) for the sharpest ~8.25ppm signal. Note that we could not access this information with WG (or indeed even with the conventional PRESS sequence), as no resonances were observed in the downfield region via these MRS methods. Importantly, in phantoms, no differences whatsoever were observed in T1 between the SE-SLR and WG sequences for non-exchanging systems (not shown). These measurements clearly demonstrate the presence of a T1 relaxation enhancement effect in *ex-vivo* tissues. Moreover, since the upfield metabolic resonances in Figure 3 correspond to non-labile ^{1}Hs, the direct mechanism for the phenomenon cannot be attributed to exchange and hence is reflecting some form of cross-relaxation. We have also been able to detect a remarkable signal increase of downfield metabolites upon spectrally-selective excitation, which can be reasonably expected to arise mostly from exchange effects. From a practical perspective our findings open the possibility of performing relaxation-optimized experiments[4] in tissues leading to improved spectra. Furthermore, understanding these effects may lead to new sources of contrast in both diseased and normal tissues –where differences in structural integrity are likely to affect the phenomenon. That different metabolites exhibit different T1 modifications may also lead valuable insight into metabolic compartamentalization. Despite requiring a minimum TE of ~40ms, the spectra obtained can be easily J-edited and extended towards other resonances; since the metabolite T2s are on the order of hundreds of milliseconds even in high fields[2], the non-water-suppressed scheme seems to offer significant advantages[9]. Further studies are needed to extend these findings to *in-vivo* systems, to other resonances, and to elucidate the mechanisms by which T1 relaxation enhancement occurs in both the exchanging downfield region and the non-exchanging upfield region.

Conclusions. It has been unambiguously shown that effective metabolic T1s in tissues will depend on how resonances are excited. Most studied metabolites show statistically significant relaxation enhancement properties upon selective-excitation, affording a straightforward way to enhance MRS's sensitivity per unit time. Equally important, these effects open new routes to investigate the nature of metabolic interactions among them and with water in tissues, at a molecular level.

References. [1] Cadalbu C et al., Magn. Reson. Med. **62** (2009) 862. [2] De graaf RA et al., Magn. Reson. Med. **56** (2006) 386. [3] Pervushin K et al., J. Am. Chem. Soc. **124** (2002) 12898. [4] Schanda P and Brustcher B, J. Am. Chem. Soc. **127** (2005) 8014. [5] MacMillan EL et al., Magn. Reson. Med. **65** (2011) 1239. [6] Kruiskamp M.J., et al., NMR Biomed. **14** (2001) 1. [7] Pauly J et al., IEEE Trans. Med. Imaging. **10** (1991) 53. [8] Piotto et al., J. Biomol. NMR., **2** (1992) 661. [9] Dong Z, et al., Magn. Reson. Med. **55** (2006) 1441.