In vivo lactate T1 and T2 relaxation measurements in breast tumors using SS1-SelMQC editing sequence

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Introduction: Multiple quantum (MQ) editing techniques have been developed for lactate (Lac) detection with complete suppression of water and lipid resonances in a single scan (1). Recent studies on prostate cancer and breast tumor have shown changes in Lac level in tumor tissues (2-3). The presence of fat and water in high concentration makes it difficult in observing and quantifying Lac. The measurement of T_1 and T_2 is essential for absolute quantification of Lac to differentiate benign and malignant tumor. We measured Lac with improved lipid and water suppression using 1331 binomial composite Spectral-Selective Pulses in Selective MQ Coherence (SS1-SelMQC) compared to the original SS-SelMQC(1) sequence and T_1 and T_2 of Lac been determined by incorporating T_1 and T_2 variables in SS1-SelMQC (T_1-SS1-SelMQC and T_2-SS1-SelMQC). Here we report T_1-SS1-SelMQC and T_2-SS1-SelMQC sequence to measure in-vivo Lac T_1 and T_2. We have standardized our pulse sequences with phantom studies and demonstrated in nude mice implanted with MCF-7, BT-474, MDA-MB-231 and MDA-MB-435 breast tumors. Invivo T_2 of Lac was found to be significantly different in prognostic molecular markers.

Materials and Methods: All MR imaging and spectroscopy experiments were performed on a 4.7 Tesla Bruker Biospin spectrometer. Animal studies were conducted in compliance with protocols approved by the Institutional Animal Care and Use Committee. MCF-7, BT-474, MDA-MB-231and MDA-MB-435 cancer cells were purchased from ATCC. Cells were grown in DME F12, DME HG for MCF-7, BT-474 respectively and RPMI for MDA-MB-231 and MDA-MB-435, and media were supplemented with 10% FBS, 1% penicillin and 1% streptomycin. The cells were incubated in 5% CO_2 atmosphere at temperature 37 °C. Once cells reach 80-90% confluence, cells were washed with PBS followed by trypsinization. The cells were then re-suspended in media (5×10^7 cells/mL). To this equal volume of Matrigel was mixed and mixture is ready for inoculation in mice body. 4-6 week old Athymic nu/nu female mice were used for the study. Two days before estrogen pellet was inserted in MCF-7 and BT-474 group mice. $5 * 10^6$ cells inoculated on the mammary fat pad of the mice. Tumor volumes were calculated using a hemi ellipsoid formula $V = (\pi/6) * x * y * z$; where *x, y* and *z* are the length, breadth and depth of the tumor respectively. Phantom studies were performed with three Lac concentrations viz., 5, 15 and 30 mM.

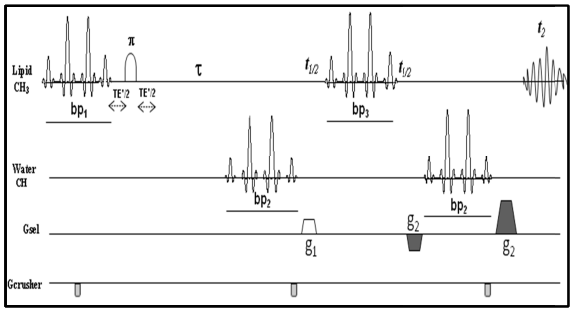

Fig1: T_2-SS1-SelMQC pulse sequence to measure T2 of Lactate

NMR Experiments Mice were anesthetized using a mixture of isoflurane (1.5 - 2.5%) air (10%) and placed inside a custom-designed MR probe. The magnet was shimmed to a half height line width of less than 50 Hz for the ^1H water signal. The non-localized spectra were acquired using T_1-SS1-SelMQC to measure T_1 of Lac and T_2-SS1-SelMQC to measure T_2 of Lac**.** The SS1-SelMQC sequence combines selective radio frequency pulses with gradient filtering to achieve Lac editing and efficient lipid and water suppression in a 'single-shot'. Frequency-selective pulses were employed to prepare MQ coherences for the Lac methyl signal. Single-quantum lipid and water resonances were then eliminated by multiple-quantum selection gradients. To ensure complete lipid suppression and a high signal-to-noise ratio for the Lac methyl signal, a two-step phase cycling (0°/180°) was applied for the selective 90° pulse at the Lac CH resonance frequency. In the T_1-SS1-SelMQC, T_1 measurement of Lac was performed with insertion of inversion 'mao4' shaped pulse with 2ms pulse width, similar to (4) and varied the inversion time before applying the SS1-SelMQC. In T_2-SS1-SelMQC, Lac T_2 relaxation was measured by Incorporating CH_3 selective 15ms single lobe 'sinc' pulse, during the MQ-preparation period of SS1-SelMQC (Fig1). This allows inserting a variable delay time TE' to measure Lac T_2 decay. The T_1 measurement was done by subtraction method (5). 1D-spectra were processed using Xwin-NMR, Bruker software. Statistical analysis was performed using SPSS software. Differences in T_1 and T_2 in molecular prognostic markers were analyzed using non-parametric Mann-Whitney U test.

Results and Discussion: T_1-SS1-SelMQC and T_2-SS1-SelMQC pulse sequences were used to measure T_1 and T_2 of Lac in phantom and *in-vivo* tumors. Representative spectra of MCF-7 of tumor volume 300mm^3 as a function of inversion time (T1) and its curve fit and echo time TE and its curve fit is shown in **Fig 2(A&B)**. The in-vitro Lac T_1 for 5, 15 and 30mM was found to be 1.34s± 0.05, 1.21s±0.002 and 1.13s±0.03 respectively. The T_2 of 5, 15 and 30 mM was 0.6s±0.02, 0.51s±0.03 and 0.50s±0.03 respectively. The measured T_1 and T_2 relaxation times of Lac in breast tumors are shown in Table 1. The T_2 of Lac was significantly different in ER PR+ Vs –, Her2+ Vs – and TN+Vs – (Table 1). The T_1 was not significantly different in these three prognostic markers. Our experimental measured T_1 and T_2 are in the range of reported T_1 and T_2 (4).

Conclusion: The reported T_1-SS1-SelMQC and T_2-SS1-SelMQC pulse sequences are effective to measure T_1 and T_2 of Lac in various tumor types. Measurement of T_2 could be used to differentiate different tumor types.

Reference: 1) Thakur SB, et al., Magn Reson Med., 62: p 591-598 (2009). 2) Inna Serganova et al, August 15, 2011; doi: 10.1158/1078-0432.CCR-11-039. 3) J.Yaligar et al, 25 May, 2011, DOI: 10.1002/nbm.1723. 4) Muruganandham M, et al., Magn Reson.Med ,52:p 902-906 (2004). 5) Kim S, et al., Magn Reson Med. 31: p 445-449 (1994).

Acknowledgement: We want to thank Dr. Mihaela Lupu and Ms.Natalia Kruchevsky for their help.
This work is supported by DOD BCRP award W81XWH-09-1-0042.

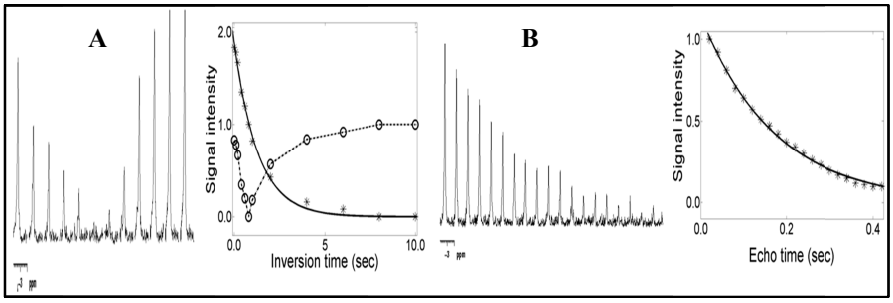

Fig 2: In vivo (MCF-7 tumor) Lac signal recovery of (A) T_1-SS1-SelMQC with variable recovery delay 0.1 to 10s. Lac signal decay (B) T_2-SS1-SelMQC with 2*TE′(0.08 to 0.42s) with 0.02 s increments. And their respective curve fits.

Table1: T_1 and T_2 of Lac in different tumors and differences in molecular prognostic factors.

Tumor	No of Mice	T_1(mean±SD) (sec)	T_2(mean±SD) (sec)
MCF-7	6	1.80±0.23	0.16±0.02
BT-474	6	1.66±0.26	0.17±0.02
MDA-MB-231	5	1.49±0.18	0.12±0.004
MDA-MB-435	4	1.87±0.23	0.12±0.009
Factor		p-Value(T_1)	p-Value(T_2)
ER,PR+/ER,PR-	9/12	0.28	0.0001
Her2+/Her2-	6/15	0.32	0.004
TP+/TP-	6/9	0.49	0.0002