In vivo Lactate T_1 and T_2 Relaxation Times in Preclinical Cancer Models – Absolute Quantification of Tumor Lactate

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Target Audience: Our *in vivo* study is of great interest to cancer researchers investigating the relationship of tumor lactate metabolism and prognosis.

Purpose: More aggressive tumors are characterized by increased glycolysis and have been associated with increased lactate production and acidity[1], with low tumor pH contributing to a suppressed T-cell immune response[2]. To be able to relate tumor lactate levels with tumor vascularity, treatment response, etc., absolute quantification is essential. While MR acquisition techniques exist to measure lactate *in vivo* noninvasively, absolute quantification is hampered by the lack of available *in vivo* lactate T_1 and T_2 relaxation times, which are difficult to acquire. For the few preclinical tumor models where data exist, T_1 and T_2 relaxation times may vary widely, depending on tumor type and field strength (**Table**)[3-6]. Here, we have measured *in vivo* lactate T_1 and T_2 relaxation times in a panel of preclinical cancer models of different aggressiveness for absolute tumor lactate quantification.

Ref	Tumor	T_1 [s]	T_2 [s]	B0 [T]
3	R3327-AT leg	1.61±0.03	0.20±0.02	4.7
4	Colon-38 sc foot	1.38	0.117	4.7
4	MCa sc foot tumor		0.068	4.7
5	C6-glioma brain	1.728±0.084	0.199±0.012	4.7
6	67NR mammary fat pad	1.61	0.21	7
6	4T1 mammary fat pad	1.86	0.34	7
*	BWH sarcoma flank	1.92	0.14	7

*Rizwan, Zakian, personal communication

Methods: *Tumor Models:* We studied to-date 2 prostate cancer (CaP) and 3 breast cancer cell lines – MycCaP (spontaneously immortalized cells from C-Myc transgenic mouse with CaP, androgen naïve[7]), RM-1 (CaP of Ras+Myc-transformed C57BL/6 mouse[8]), 4T1wt (mouse mammary carcinoma[9]) and 4T1 clones with modified lactate dehydrogenase A/B expression (LDH) expression levels[10]. All cell lines were grown in Dulbecco's Modified Essential Medium, supplemented with 10% fetal bovine serum, 100 U/ml Penicillin and 100 µg/ml Streptomycin, and, where applicable puromycin, at 37 °C in 5% CO_2. Prostate cancer cells were injected subcutaneously in the right flank of NOD/SCID mice, while breast cancer cells were orthotopically implanted into the mammary fat pad of Balb/C mice.

In Vivo MR: The MR experiments were performed using a custom-built, solenoid ^{1}H MR coil on a horizontal-bore Bruker 7T magnet. The mice were anesthetized with < 2% isoflurane in oxygen, their breathing rate kept at 50-90 breaths/min, and their core temperature maintained at 34-37°C. After tumor positioning, tuning and matching of the ^{1}H MR coil, the water line width was optimized to ~30-70 Hz full-width-half-maximum by field map-based shimming. Tumor lactate was measured using new implementation of the SelMQC[11] and SelMQC-based T_1 and T_2 acquisition[4] sequences that feature enhanced FOV and slice selection, plus slice, global, and 2D MRSI acquisition. *In vivo* lactate sample spectra for the T_1 and T_2 relaxation time measurements are shown in **Fig. 1**. To measure tumor lactate, whole-tumor, single-slice MRS and MRSI data were acquired with 16 mm x 16 mm field-of-view, 8x8 matrix, 90° excitation, 3 s TR, 120 ms TE, with varying slice thickness to cover the entire tumor. Data processing and lactate signal fitting was done using XsOsNMR. We used the phantom replacement method[6] for the absolute quantification of lactate. We calculated the lactate concentration **[Lac]** by correcting signal intensities **S** for differences between tumor and phantom (subscripts $_T$ and $_{Ph}$, respectively) in number of spectral averages (**na**), coil loading (**Q**), sample volume (**V**) and relaxation times (**T_1, T_2**): $[Lac_T] = [Lac_{Ph}] \bullet S(Lac_T)/S(Lac_{Ph}) \bullet na_{Ph}/na_T \bullet V_{Ph}/V_T \bullet Q_{Ph}/Q_T \bullet \exp(TE(1/T_{2,T}-1/T_{2,Ph})) \bullet (1-\exp(-TR/T_{1,Ph}))/(1-\exp(-TR/T_{1,T}))$ with **TE**: echo time, **TR**: relaxation time.

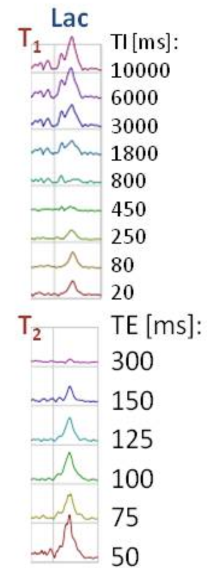
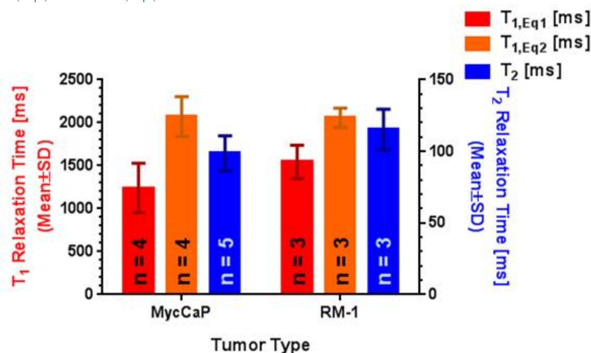


Fig. 1: *In vivo*, fully relaxed lactate MR spectra, acquired with the indicated inversion (TI) and echo (TE) times.

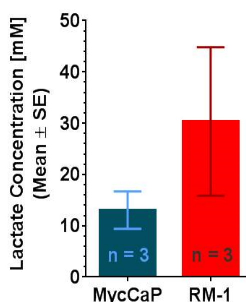
Results & Discussion: The T_1 and T_2 data acquisition parameters and analysis were optimized on MycCaP and RM-1 tumors. We found using the lactate signal height to calculate T_1 and T_2 to be the most expedient. In fatty tumors with low lactate levels, residual lipid contamination, due to imperfect multiple quantum filtering, can significantly contribute to the signal area obtained by integrating a spectral region. Using spectral fitting to deconvolve residual lipid from lactate is time consuming and does not improve the T_1 and T_2 data fitting (data not shown). To calculate T_1 from our magnitude spectra (**Fig. 1**), we compared fitting of the magnitude data by **Eq1:** $S = M_0 \bullet (1-e^{-TI/T1})+M_{z0} \bullet e^{-TI/T1}$, where which data points to be inverted was decided by best fit, to fitting of unaltered magnitude data with **Eq2:** $S = |M_0 \bullet (1-e^{-TI/T1})+M_{z0} \bullet e^{-TI/T1}|+A$). Compared to Eq1, fitting by Eq2 resulted in larger fitting errors (data not shown), as well as significantly higher T_1 relaxation times (**Fig. 2**). This was due to the inability of Eq2 to fit S=0 (crossover / turning point) reliably with the limited number of data points available around the turning point, resulting in **A >> 0** (**Fig. 1**). Lactate T_2 data were fitted using $S = M_0 \bullet e^{-TE/T2}$. Though MycCaP tumors tended to have smaller T_1 and T_2 relaxation times than RM-1, these differences were not significant (**Fig. 2**). None of the prostate tumors showed significant necrosis on MRI. Preliminary results for the absolute tumor lactate concentration in prostate tumors are shown in **Fig. 3**. Relaxation time acquisitions and absolute quantification of tumor lactate for the breast cancer models is in progress. When compared to the literature T_1 and T_2 values (**Table**), our CaP results indicate that differences in relaxation times between tumors may be more strongly impacted by tumor location than tumor type. Our data will inform us if in small tumors without extensive necrosis, as studied here, lactate T_1 and T_2 relaxation times vary significantly between tumor types and location.

Fig. 2: Average T_1 and T_2 relaxation times for 2 murine CaP models. Paired t-Test: $T_{1,Eq1,MycCaP} < T_{1,Eq2,MycCaP}$, P = 0.0419; $T_{1,Eq1,RM-1} < T_{1,Eq2,RM-1}$, P = 0.0770.



Conclusion: We now have the ability to reproducibly acquire lactate T_1 and T_2 relaxation times *in vivo* in a variety of tumor models for future research into the role of lactate metabolism in tumor development, progression, and treatment response.

Fig. 3: Absolute Lactate levels in 2 CaP models.



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