Reproducibility of 13C magnetic resonance spectroscopy measurements with hyperpolarized [1-13C]pyruvate

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Target audience: Determination of the reproducibility of ^{13}C magnetic resonance spectroscopic measurements of hyperpolarized [1-^{13}C]pyruvate metabolism and of the factors that affect reproducibility is important for radiologists given the recent translation of this technique to the clinic [1].

Purpose: Hyperpolarized [1-^{13}C]pyruvate has shown great promise in clinical oncology, particularly in the assessment of early response to treatment, and has also been the only and first hyperpolarized substrate to be used in humans[1,2]. However, the reproducibility of serial scans has never been determined. It is important to determine whether serial changes in the kinetics of hyperpolarized [1-^{13}C]pyruvate conversion to lactate reflect the real effects of therapy or whether they are due to changes in mouse physiology and method variability. The aims of this study were to determine the reproducibility of measurements with hyperpolarized [1-^{13}C]pyruvate as a probe for detecting treatment response in tumor-bearing mice and to understand the metabolic changes responsible for the observed variability in the kinetics of pyruvate conversion to lactate.

Methods: C57BL/6 mice were implanted subcutaneously in the flank with EL-4 cells (a murine lymphoma) and divided into two cohorts: fasted (F) for 18 hours (n=9) and non-fasted (NF) (n=7). All mice were anaesthetized with isoflurane, taped to a holder and placed in a heated probe at ~37ºC. Respiratory rate and body temperature were monitored using a Biotrig physiological monitor (Small Animal Instruments, Stony Brook, NY). A 20 mm diameter surface coil (Rapid Biomedical GmbH, Rimpar, Germany) was placed over the tumors and ^{13}C-MR spectra were acquired from a 6 mm slice immediately after i.v. injection of 0.2 ml of pyruvate, using a 7 T horizontal bore magnet (Agilent, Palo Alto, CA). The hyperpolarized solutions of [1-^{13}C]pyruvate were prepared using a polarizer (Hypersense, Oxford Instruments, Oxford, UK). Each mouse was submitted to the same imaging protocol twice, with a 4 hour interval between scans. All mice were recovered from anaesthesia after the first scan, with no food provided for the fasted animals between scans. Rate constants for conversion of pyruvate to lactate (k_P) were calculated using Matlab (Mathworks). Coefficients of variation (CV) and differences in k_P values between studies in the same individual were calculated to determine reproducibility. Tumor perfusion was assessed by DCE-MRI. Blood analyses of lactate, pyruvate, glucose, corticosterone and adrenaline were performed in the two groups of mice at two different time points, corresponding to the first and second scans. Tumors from these mice were removed, freeze clamped and extracted using perchloric acid for NMR analysis of lactate and alanine concentrations.

Results: The CV for the mean k_P was 12±10% for the F mice and 37±26% for the NF mice. The mean percentage change in the k_P value observed between the first and the second scan was 91% in the NF mice, and 14% in the F animals. A significant difference in the mean k_P, determined from the first scan, between the F and NF cohort was noted, as well as between the first and the second scan of the NF mice (Fig.1). Blood measurements revealed a significant increase in lactate concentration between the first and second scan in the NF mice. Blood pyruvate was also significantly different in the first scan between the F and NF animals. Blood corticosterone levels were globally higher in the F mice and adrenaline showed an increasing trend between scans in both F and NF groups. Moreover, tissue analysis showed a significant difference in lactate levels at the time of the first scan between the F and NF animals. No changes in perfusion were observed between groups. Blood glucose levels, time of injection, tumor size, and body weight did not appear to contribute to the variability of the scans.

Discussion: Low variability in the kinetics of hyperpolarized[1-^{13}C]pyruvate conversion to lactate was observed in fasted mice. This might be due to the similar blood and tumor lactate levels observed between scans. In NF mice, however, significant changes in the k_P and the lactate levels were observed between scans, possibly as a consequence of the release of stress-induced hormones before the second scan, as shown by the trend of increasing levels of these hormones between scans. The observed differences within and between groups were not explained by changes in perfusion or glucose levels.

Conclusion: Hyperpolarized[1-^{13}C]pyruvate is a reproducible probe of tumor treatment response, showing relatively little variability in the kinetics of its conversion to lactate when injected into fasted mice. These results suggest that the fasted state may be more optimal for performing this technique in human patients.

References: [1]Nelson SJ (2012) Proc. Intl. Soc. Mag. Reson. Med. 20, Melbourne, Australia; [2]Day SE (2007) Nat Med 13:1382.

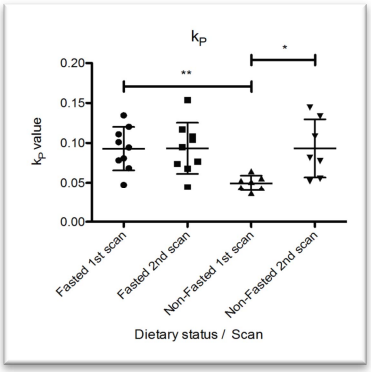

Figure 1- k_P values of each animal from each group (Fasted and Non-Fasted) and scan (first and second scan).