Hyperpolarized ^{13}C-alpha-ketobutyrate, a pyruvate analog

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Introduction- Lactate dehydrogenase (LDH) is characterized by relatively broad substrate specificity[1]. For example, α-ketobutyrate (αKB) is an endogenous structural analog of pyruvate with low acute toxicity that is also readily reduced via LDH, to α-hydroxybutyrate (αHB, Fig. 1). αKB differs biochemically from pyruvate, however, in potentially interesting ways. In particular, the activity of LDHA expressed M protein subunits (LDH-5 like isoenzymes) with αKB is highly attenuated relative to pyruvate, while activity of LDHB expressed H protein subunits (LDH-1 like isoform) with αKB is nearly equivalent[2], suggesting that HP αKB could be a useful probe of LDHB expression and LDH-1 like isoform (e.g. heart or kidney LDH). Relative LDH activity of serum with αKB vs. pyruvate has previously been used clinically to measure LDH isoenzyme fractions to diagnose myocardial infarction[3]. Other differences from pyruvate include: 1) differential utilization by alternate pathways[4] (i.e. different route of entry into Krebs cycle), 2) differences in vascular permeability and/or cellular transport[5], and 3) different endogenous pool sizes. The purpose of this study was to show feasibility of hyperpolarizing ^{13}C-αKB and detecting its rapid enzymatic conversion via LDH *in vivo*, and investigate the results in comparison with HP ^{13}C-pyruvate.

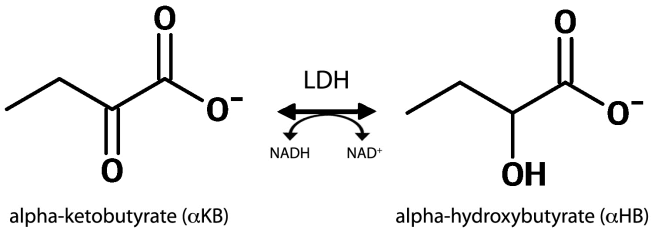

Fig. 1. Redox reactions of αKB / αHB catalyzed by LDH

Methods- A sodium salt of [U-^{13}C]αKB (CIL, Andover, MA) was dissolved in 50/50 glycerol/water, mixed with trityl radical (15mM) and Gd-DOTA (1.0mM), and then polarized via dissolution DNP. Aqueous T_1's were measured at 3T and found to be similar to pyruvate (50s for C_1, 40s for C_2). For a paired *in vivo* comparison of αKB and pyruvate, three rats were scanned by back-to-back slab-localized MRS of HP [U-^{13}C]αKB and HP [1,2-^{13}C]pyruvic acid, for each of two different concentrations (40mM and 10mM, 2.5mL infusions via tail vein). For both compounds, doublets were observed for C_1 resonances due to coupling to co-labeled C_2. Scans were separated by one hour. A 5.5cm axial slab covering liver and kidneys was repeatedly excited (20°) every 3s over 30s (pulse bandwidth=2.8kHz, center ~178ppm).

Results and Discussion- Rapid *in vivo* conversion of HP αKB to HP αHB via LDH was detected in the spectra of all rats. The mean summed αKB-to-αHB ratio after 40mM HP αKB infusion was 5.2 ± 0.6 times the HP pyruvate-to-lactate ratio after 40mM HP pyruvate infusion in the same animals. Unlike HP pyruvate spectra, no transamination product (i.e. α-aminobutyrate) was observed after HP αKB infusions, and only a very small degree of decarboxylation by PDH was detected[4], by the appearance of a small HP [^{13}C]bicarbonate peak in some of the data. The 10mM infusions resulted in a more variable relationship between the αKB and pyruvate data. While both pyruvate-to-lactate and αKB-to-αHB ratios were in all cases higher with 10mM infusions (by a factor of ~3), the increases were not consistent by probe. The αKB-to-αHB ratio increased by 2.2-fold more than the pyruvate-to-lactate ratio in one rat, but in the other two rats actually showed smaller increases than the pyruvate-to-lactate ratio. Changes in these HP ratios as a function of concentration could reflect varying reactivity of αKB with LDHA vs. LDHB expression *in vivo*, such as occurs *in vitro*[1-3]. This is because LDHB expressed H subunits have much lower K_m values (~1.0mM or less) for reducible substrates than LDHA expressed M subunits, a feature which is thought to contribute to their functional role in the oxidation of lactate as an energy source[6] (for instance by myocardium and renal cortex). However, these limited initial results may be confounded by potential effects of enzyme saturation and vascular signal contributions, necessitating further experiments.

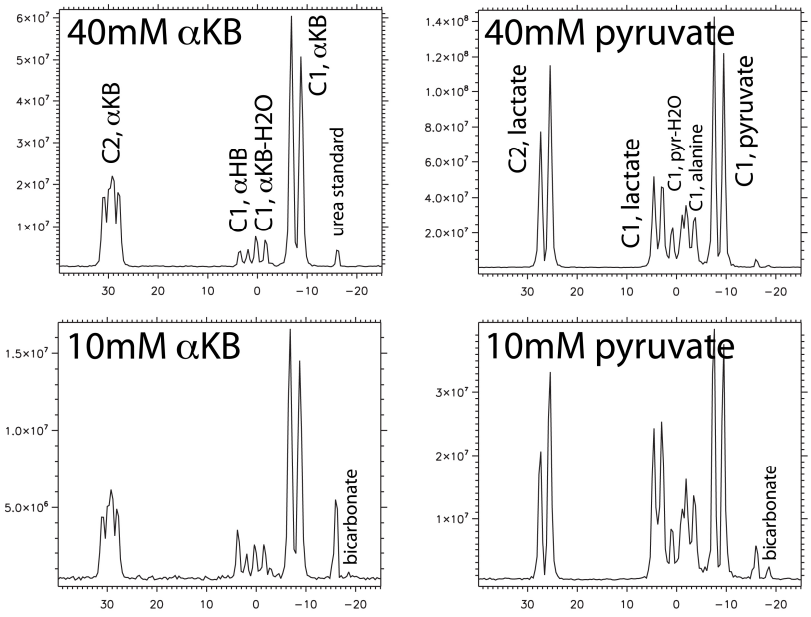

Fig. 2. In vivo slab spectra (magnitude) from a single rat for HP [U-^{13}C]aKB and HP [1,2-^{13}C]pyruvate for experimental conditions as described in text, summed over all dynamic time points.

Conclusion- We have demonstrated the hyperpolarization and rapid *in vivo* enzymatic conversion of an endogenous structural analog of pyruvate, [^{13}C]αKB, via LDH. Based on prior *in vitro* work and our initial results, this HP probe may exhibit useful selectivity for LDHB expression and the associated activity of LDH1 like isoenzymes *in vivo*.

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References- 1. Kim and Whitesides. *JACS*. 1988. 2. Plummer et al. *Biochem J*. 1963. 3. Rosalki and Wilkinson. *JAMA*. 1964. 4. Lapointe and Olson. *Arch Biochem Biophys*. 1985. 5. Halestrap. *IUBMB Life*. 2011. 6. Markert. *Cell Biochem & Function*. 1984.