Use of Hyperpolarized Carbon-13 Magnetic Resonance For Non-Invasive Observation of Metabolic Regulation

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Introduction

Hyperpolarization of ^{13}C-labelled metabolic substrates provides the MR signal necessary to visualize substrate uptake and metabolism in real-time, *in vivo* [1, 2]. To date, hyperpolarized MR has only been used to report on the current metabolic state in a given tissue, and has yet to provide any information regarding the nature of metabolic regulation. Changes that govern substrate selection are often driven by changes in enzyme activity and protein expression and are generally assessed with invasive biochemical assays, which require *in vitro* tissue samples.

The purpose of this study was to demonstrate that hyperpolarized MR could be used, not only to reveal metabolic fluxes in a given metabolic state, but to identify mechanisms of metabolic regulation under various conditions. This proof-of-concept work focused on regulation of the enzyme pyruvate dehydrogenase (PDH) which controls the oxidation of pyruvate into acetyl CoA. PDH flux can be inhibited by both reduced activity of the PDH enzyme or by end product inhibition due to excessive levels of acetyl CoA. In this work we aimed to differentiate the dominant mechanism of PDH regulation in the fed and fasted states by co-infusing hyperpolarized [1-^{13}C]pyruvate with malate, a Krebs cycle intermediate. Increased levels of malate should increase Krebs cycle flux, reducing the level of acetyl CoA and thereby removing end product inhibition on PDH.

Methods

Six male Wistar rats were examined with the hyperpolarized MR protocol, detailed below, on four separate occasions. Rats were examined in both the fed and fasted states (to modulate PDH activity [3]), with either hyperpolarized [1-^{13}C]pyruvate alone or with hyperpolarized [1-^{13}C]pyruvate co-infused with malate (to increase Krebs cycle flux and acetyl CoA uptake).

In vivo results were verified *in vitro* using a ^{1}H NMR based metabolomics approach. The four metabolic states that were studied in living rats were replicated in a separate group of rats and cardiac tissue was harvested and freeze clamped 30 s after tracer infusion, to correspond with the time of MR data acquisition. The concentration of Krebs cycle intermediates in cardiac tissue extracts were compared in the presence and absence of infused malate, in both fed and fasted rats.

Hyperpolarized MR Protocol: [1-^{13}C]pyruvate was hyperpolarized and dissolved as previously described [3]. The resultant 80 mM hyperpolarized tracer was diluted to 40 mM with Krebs Henseleit (KH) buffer. For malate co-infusion experiments, the KH buffer also contained 80 mM malate, such that the resultant solution contained 40 mM [1-^{13}C]pyruvate and 40 mM malate. The hyperpolarized tracer was infused into the rats in a 7 T MR scanner and cardiac spectra were acquired with a surface coil every 1 s for 1 min. Conversion of pyruvate to $H^{13}CO_3^-$ was monitored and $H^{13}CO_3^-$/pyruvate ratio was used as a marker of PDH flux.

In Vitro Analysis: Metabolites were extracted from frozen tissue and high resolution ^{1}H NMR experiments were performed in an 11.7 T Bruker spectrometer with a 5 mm probe. To assess which spectral regions were responsible for significant differences between groups a univariate data analysis procedure was used and NMR spectra were assigned with reference to the literature.

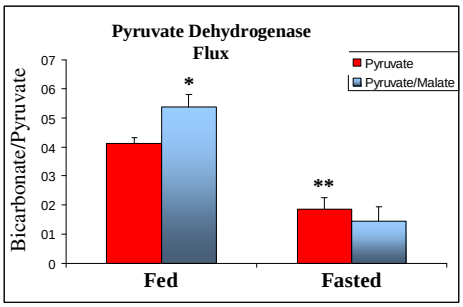

Figure 1 *Observed $H^{13}CO_3^-$/pyruvate ratio for fed and fasted rats, infused with pyruvate alone and pyruvate plus malate. *p<0.02, **p<0.002.*

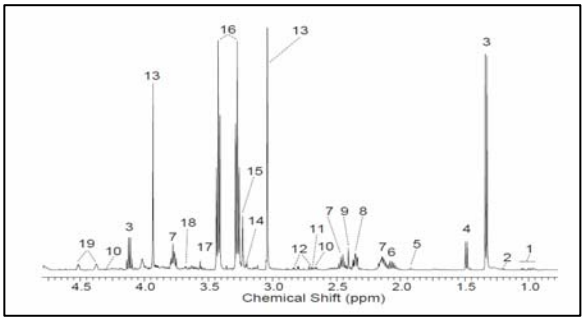

Figure 2 *^{1}H NMR spectrum of cardiac tissue extract. Peak 9: succinate, peak 10: malate, peak 11: citrate.*

Results

In fed rats, infusion of malate increased $H^{13}CO_3^-$/pyruvate by 32% compared with pyruvate alone, indicating that removal of acetyl CoA by its incorporation into the Krebs cycle increased PDH flux (Figure 1, *p<0.02*). PDH flux was 54% lower in fasted rats injected with pyruvate alone compared with fed rats (*p<0.002*) and did not change due to malate co-infusion. Here, low PDH activity appeared to prevent additional enzyme flux.

Fed rats which received a pyruvate/malate co-infusion showed a significant, 57% increase in the region of the NMR spectrum that represents malate, compared with fed rats infused with pyruvate alone (*p=0.02*). This result confirmed that malate was taken up into cardiac myocytes. Additionally, the other Krebs cycle intermediates detectable with this NMR protocol showed a trend to increase upon malate infusion. Succinate was significantly increased in concentration by 21% (*p=0.01*), while the region associated with citrate was non-significantly elevated by 25% (*p=0.06*). This result confirmed that malate was also incorporated into the Krebs cycle and increased overall carbon flux. In fasted rats, no difference between pyruvate and pyruvate/malate groups was observed for any metabolite.

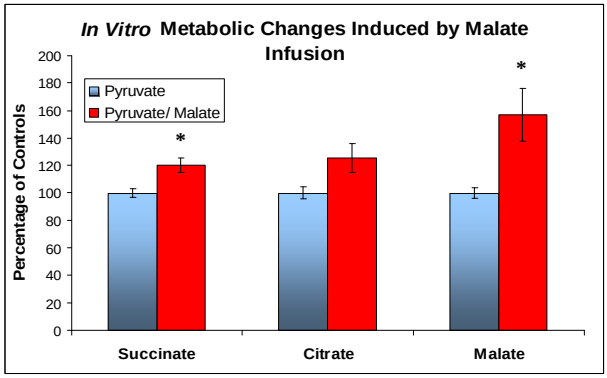

Figure 3 *The percentage change to each metabolite between the fed pyruvate and pyruvate plus malate groups. *p<0.05.*

Discussion

In vivo results suggested that in fed rats, end-product inhibition acutely controlled PDH flux, while in fasted rats, PDH activity limited pyruvate oxidation; these mechanisms were confirmed by *in vitro* experiments. Therefore, by performing experiments with both a hyperpolarized tracer alone and a hyperpolarized tracer co-infused with another metabolite, we were able to distinguish between *in vivo* mechanisms of enzymatic regulation. This strategy may be applied to explore more complicated signaling pathways, given an understanding of the mechanisms being investigated and strategic development of a multi-component hyperpolarized tracer. Additionally, the co-infusion method manipulated systemic metabolism such that [1-^{13}C]pyruvate could report on flux through both PDH and the Krebs cycle. This approach represents a simple method for using [1-^{13}C]pyruvate to indirectly derive information about multiple metabolic pathways, until further hyperpolarized agents are available.

References

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