Real Time Assessment of Krebs Cycle Metabolism with Hyperpolarized [2-^{13}C]Pyruvate

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Introduction

The Krebs cycle plays a fundamental role in cardiac energy production and is often implicated in the energetic imbalances characteristic of heart disease. Previous measurements of Krebs cycle metabolism have used carbon-13 MR spectroscopy (MRS) combined with isotopomer analysis[1] but these techniques have generally been limited to steady-state experiments. Metabolic imaging with hyperpolarized ^{13}C MRS has enabled unprecedented visualization of real-time mechanisms of normal and abnormal metabolism[2]. However, no hyperpolarized MR tracer, or other experimental technique, has routinely monitored instantaneous Krebs cycle metabolism in whole organs. The aim of this work was to use hyperpolarized [2-^{13}C]pyruvate as an alternative metabolic tracer to enable the direct monitoring of Krebs cycle metabolism in normal and ischaemic hearts.

Methods

[2-^{13}C]pyruvate was hyperpolarized in a HyperSense system (Oxford Instruments, UK) according to the method of Ardenkjaer-Larsen[3]. Six rat hearts were perfused in the Langendorff mode and placed in the bore of an 11.7T vertical bore MR scanner. Hyperpolarized [2-^{13}C]pyruvate was infused while the heart was functioning normally and spectra were acquired with 1s temporal resolution. A 10 min period of no-flow ischaemia was then initiated. A second dose of the same hyperpolarized tracer was infused immediately upon reperfusion. Peaks arising from hyperpolarized [2-^{13}C]pyruvate were identified using high resolution ^{13}C NMR and by examination of cross-peaks arising from 2D ^{1}H/^{13}C NMR on tissue extracts. Whole heart cardiac ^{13}C MR spectra were analyzed using the AMARES algorithm[4] and the results plotted against time to generate metabolic progression curves, as previously described[5]. The area under the metabolic progression curves for each metabolite was then compared between healthy and post-ischaemic states.

Results

[2-^{13}C] pyruvate was found to polarize similarly to [1-^{13}C]pyruvate and was successfully dissolved to provide polarization levels greater than 20%. The relaxation time was measured to be 34s, slightly reduced when compared to [1-^{13}C]pyruvate at 45s. Figure 1 shows an example metabolic time course over 60s of spectral acquisition in a healthy heart. The appearance and subsequent decay of [2-^{13}C]pyruvate can be seen at 205ppm. Peaks visible with 1s resolution were attributed to [5-^{13}C]glutamate, [2-^{13}C]citrate, [1-^{13}C]acetyl-carnitine, [1-^{13}C]pyruvate, [2-^{13}C]pyruvate-hydrate, [2-^{13}C]lactate and [2-^{13}C]alanine. In the post-ischaemic heart, the [2-^{13}C]lactate peak was seen to increase by 70% and the [5-^{13}C]glutamate and [2-^{13}C]citrate peaks were reduced by 43% and 41% respectively (Figure 2), when compared with the healthy state.

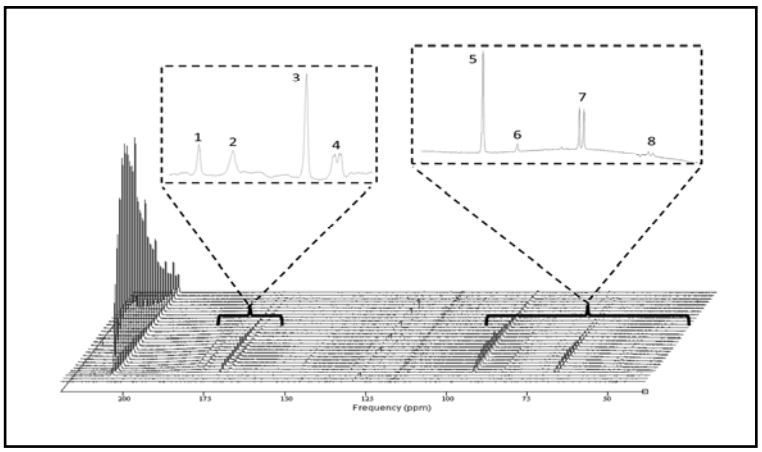

Figure 1 – Example metabolic course following infusion of [2-^{13}C]pyruvate. Peaks attributed to (1) [5-^{13}C]glutamate, (2) [2-^{13}C]citrate, (3) [1-^{13}C]acetyl-carnitine, (4) [1-^{13}C]pyruvate, (5) [2-^{13}C]pyruvate-hydrate, (6) impurity, (7) [2-^{13}C]lactate and (8) [2-^{13}C]alanine could be observed with 1s temporal resolution.

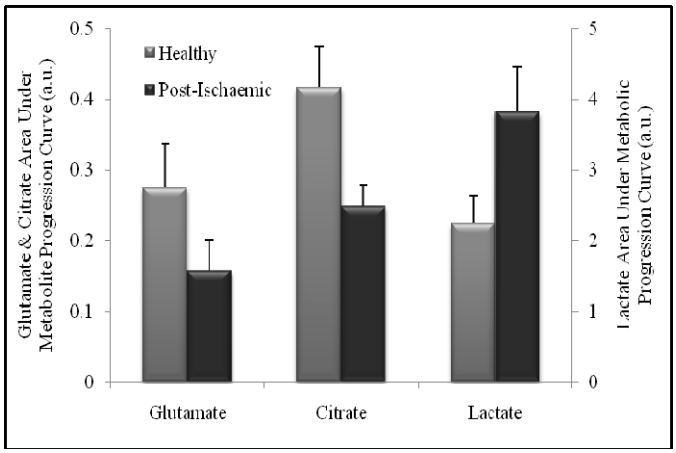

Figure 2 – Area under metabolic progression curves for glutamate, citrate and lactate. Both glutamate and citrate show a significant decrease post-ischaemia relative to the healthy heart in comparison to lactate which shows a significant increase ($p<0.05$).

Discussion

These results demonstrate the first example of direct monitoring of instantaneous Krebs cycle metabolism. The entry of [2-^{13}C] pyruvate into the Krebs cycle has been monitored with 1s temporal resolution and been shown to reveal differences between the healthy and the post-ischaemic heart. [2-^{13}C]pyruvate may have great potential in assessment of impaired metabolism in heart disease.

References

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