Measuring In Vivo Myocardial Substrate Competition Using Hyperpolarized 13C Magnetic Resonance

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INTRODUCTION: Cardiac energy is derived primarily from lipids and to a lesser extent carbohydrates and ketones. Cardiac dysfunction is often associated with a shift in substrate preference, but current *in vivo* techniques only provide direct information on substrate uptake. Hyperpolarization of ^{13}C substrates enables the measurement of cardiac metabolism with high sensitivity and specificity [1,2]. The possibility of assaying substrate selection *in vivo* would constitute a fundamental advance in the diagnosis and treatment of heart failure. The aim was to assess, *in vivo* and in real time, the competition between carbohydrate and fatty acid utilization in the rat heart using co-administration of hyperpolarized (HP) [1-^{13}C]pyruvate and [1-^{13}C]butyrate, and the specificity of the protocol was tested using a feed/fast paradigm.

METHODS: Sodium [1-^{13}C]pyruvate and sodium [1-^{13}C]butyrate with a TEMPO concentration of 33 mM in a 1:2 mixture of d_6-EtOD/D_2O were dynamically polarized for 2.5 hr in a 5T DNP polarizer. After dissolution, 1.0 mL of the hyperpolarized solution was infused into the animal within 7 s. Six groups of animals were studied. Each group was injected with HP butyrate (n = 12), pyruvate (n = 10) or both (n = 14) and the animals were fed or fasted overnight. Cardiac triggered measurements were carried out on a 9.4 T animal scanner using a ^{13}C surface coil, positioned over the chest, with 30° adiabatic RF pulses applied every 3 s with ^{1}H decoupling. NMR spectra were summed and fitted to Lorentzian line shapes to obtain ratios of observed metabolites. Error bars indicate ± standard error of the mean.

RESULTS AND DISCUSSION: **In the fed state**: HP pyruvate metabolism led to the detection of lactate, alanine, $^{13}CO_2$, ^{13}C bicarbonate and metabolite ratios are consistent with previous studies[1]. HP butyrate metabolism showed ^{13}C labeling in glutamate, β-hydroxybutyrate (BHB), citrate, acetoacetate, and acetylcarnitine. The heart is not a ketogenic organ, however, mitochondrial pseudoketogenesis [3] is a mechanism by which the ^{13}C label can be exchanged between ketones without net synthesis of either acetoacetate or BHB. True ketogenesis would result in labeling of [3-^{13}C]acetoacetate, which was never observed. Acetylcarnitine was approximately twice the intensity of the glutamate and acetoacetate peaks (Fig. 1A) and showed nearly identical intensities between the fed and fasted state (Fig. 2B), and was therefore chosen as an internal normalization (Fig. 2C). The competition presented by butyrate results in a significant decrease in the bicarbonate signal (Fig. 2A). From the perspective of butyrate metabolism, the co-injection of pyruvate produced multiple changes in the fed state (Fig. 2C). The glutamate to acetylcarnitine decreased significantly and the appearance of acetoacetate was nearly quenched. The acetylcarnitine increase and decrease in glutamate and acetoacetate signals were modulations due to competition from pyruvate for the limited number of free CoA units in the mitochondria, and the antiporting of acetoacetate when pyruvate enters the mitochondria.

In the fasted state: In addition to a change in enzyme expression in the fasted myocardium, there is a different profile of circulating substrates. The ratio of bicarbonate to alanine was markedly reduced and a significant increase in the lactate to alanine ratio was observed for pyruvate injections alone (Fig. 2A), interpreted as an increase in the redox state. In the butyrate control, the glutamate to acetylcarnitine ratio was significantly lowered (Fig. 2C), while pool sizes remain unchanged, indicating a decrease in ^{13}C labeling. A significant increase of the ketone body acetoacetate was observed (Fig. 2C), due to increased endogenous acetoacetate. With increased circulating ketones, label exchange into acetoacetate is facilitated, thereby decreasing the fraction of label available for production of [1-^{13}C]acetylCoA and [5-^{13}C]glutamate. Co-injection resulted in changes to the downstream metabolites of both substrates compared to the fed animals. The ratio of acetylcarnitine to alanine rose and a drop in bicarbonate to alanine ratios was observed. From the perspective of butyrate metabolism, the co-injection of pyruvate decreased the glutamate to acetylcarnitine in the fasted state (Fig. 2C). The change in glutamate to acetylcarnitine ratio decreased significantly upon introduction of pyruvate. Additionally, the co-injection of pyruvate in fasting results in an apparent restoration of the acetoacetate signal.

CONCLUSION: The combination of hyperpolarized ^{13}C technology and co-administration of two separate imaging agents enables noninvasive and simultaneous monitoring of both fatty acid and carbohydrate oxidation in the heart *in vivo* in a single experiment.

References:[1]M.Schroeder *et al*, PNAS (2008) [2] D.Ball *et al*, MRM (2013)[3]Fink *et al*, JBC(1988) Acknowledgements: Supported by the SNF (grant PP00P2_133562 and 31003AB_131087), the CIBM of the UNIL, UNIGE, HUG, CHUV, EPFL, and the Leenaards and Jeantet Foundations and grants CPRIT RP-101243, NIH 5 R37 HL34557, and NIH-NIBIB 5 P41 EB015908.

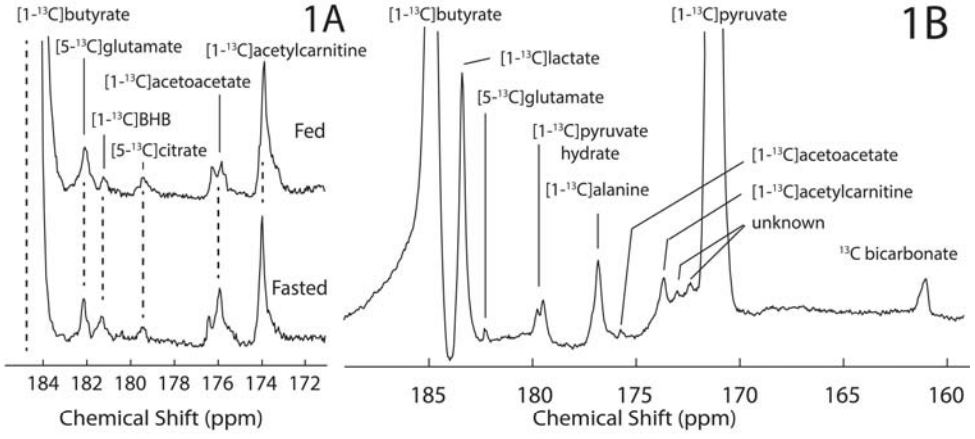

Fig 1. In vivo spectra of HP butyrate metabolism in the fed and fasted heart (A) and resulting metabolism following the co-administration of both HP butyrate and pyruvate (B).