On the Utility of Propionate as a Probe of Myocardial Energy Metabolism Using Hyperpolarization – Effects on Anaplerotic Flux and Substrate Preference

Mukundan Ragavan[1], Xiaorong Fu[1], Shawn C Burgess[1], and Matthew E Merritt[1]
[1]*Advanced Imaging Research Center, University of Texas Southwestern Medical Center, Dallas, Texas, United States*

Target Audience
The results presented in this abstract will be of interest to researchers involved in understanding energy metabolism in treatment of different myocardial pathologies and to those applying hyperpolarized imaging techniques to the heart.

Purpose
Degradation of amino acids such as isoleucine, methionine and valine and breakdown of odd chain fatty acids (FAs) yield propionyl-CoA[1] which is readily converted to succinyl-CoA. This conversion provides anaplerotic flux into the TCA cycle maintaining proper intermediate pool sizes for its correct function. Propionate, a three carbon short chain FA, is avidly consumed by the heart and can be used to probe anaplerotic flux and substrate preference in healthy and failing hearts using hyperpolarization (HP) and NMR spectroscopy.

Methods
C57BL/6 mice were used in this study. The heart was excised from the mouse and perfused in Langendorff mode with krebs-henseleit (KH) buffer (pH 7.4) containing [1,6 – $^{13}C_2$] glucose, [U-^{13}C] fatty acids and varying concentration of [1-^{13}C] sodium propionate for 30 minutes and subsequently freeze clamped. For isotopomer analysis, the frozen hearts were subjected to a perchloric acid extraction procedure. All ^{13}C NMR spectra were measured on a Bruker NMR spectrometer equipped with a cryogenically cooled probe at a magnetic field of 14.1 T.

Results and Discussion
Even small concentrations (0.25 mM) of sodium propionate in the perfusate produces a significant effect on myocardial TCA cycle pool sizes as evidenced by the ^{13}C spectra shown in Figure 1a. Presence of propionate leads to the increase in pool sizes of 4 – carbon intermediates (e.g. ~30-fold increase in the malate concentration, Figure 1b) as well as decrease in glutamate concentration in the heart without affecting cardiac function as measured by the oxygen consumption. Upon injection of HP [1 – ^{13}C] pyruvate to the perfused heart, bicarbonate production was significantly enhanced in the presence of propionate (Figure 1c).

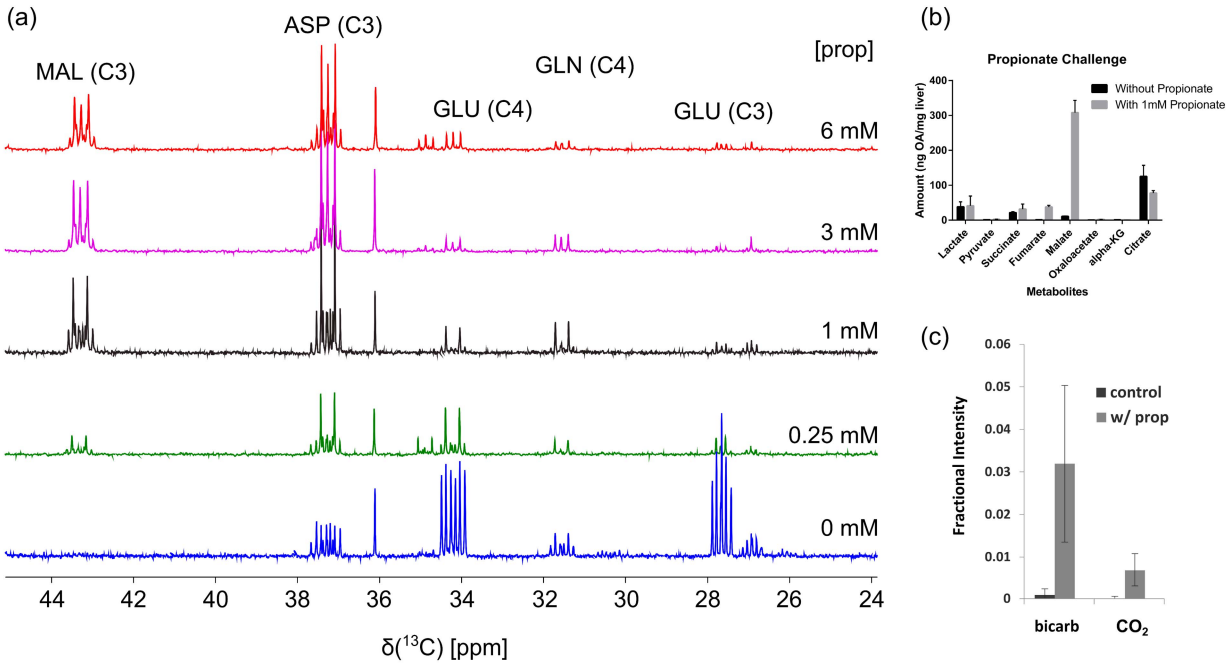

Figure 1: (a) ^{13}C spectra of PCA extracts of hearts perfused with increasing concentrations of [1-^{13}C] sodium propionate. Changes in the glutamate and malate pool sizes can be is clearly shown. (b) Histogram showing a comparison in metabolite pool sizes in the heart in the absence and presence of 1 mM sodium propionate as measured by mass spectrometry. (c) Effect of propionate on fractional ^{13}C signal intensities of metabolic products of [1-^{13}C] pyruvate in a HP experiment.[2]

Conclusions
Combination of conventional NMR measurements with hyperpolarization have yielded valuable information on the effect addition of propionate has on the anaplerotic flux and substrate utilization in the heart. Propionate increases PDH flux and pool sizes of 4 – carbon intermediates but decreases glutamate pool size.

References
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