Effect of Rapid Changes in ^{13}C Pyruvate Concentration on Lactate and Alanine Pool Sizes and ^{13}C Enrichment in the Heart

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Introduction

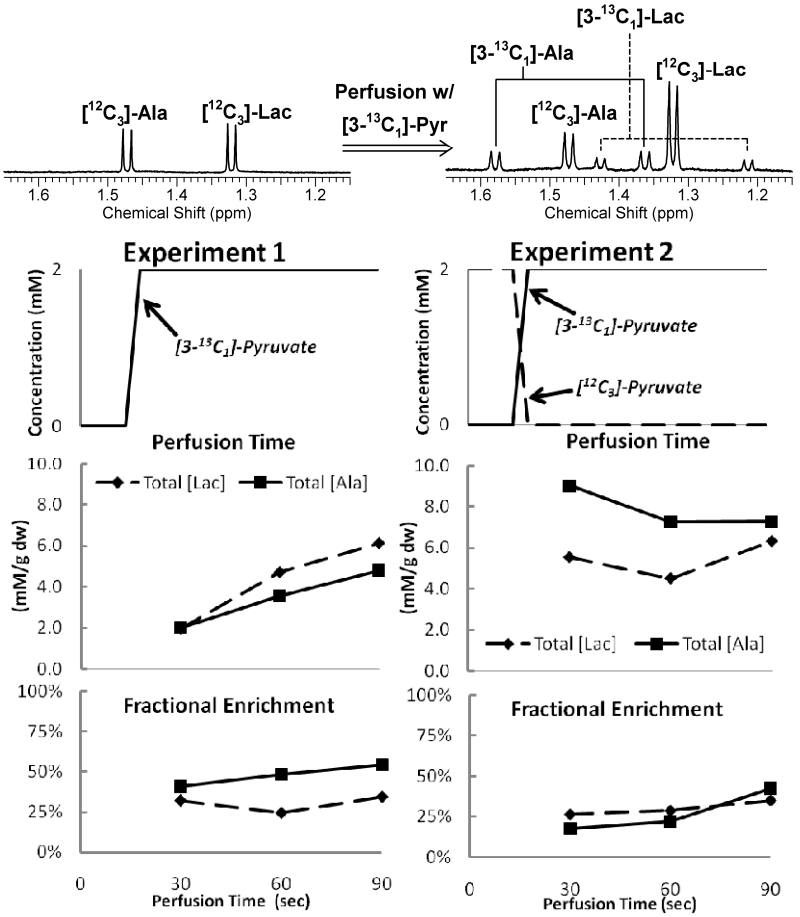
The appearance of hyperpolarized [1-$^{13}C_1$]-lactate may provide diagnostic information in metastatic cancer or the ischemic myocardium after injection of hyperpolarized (HP) [1-$^{13}C_1$]-pyruvate. The amount of [1-$^{13}C_1$]-lactate in tissue, of course, is the product: (fractional enrichment) * [lactate]. This simple relationship means that the appearance of HP-[1-$^{13}C_1$]-lactate, neglecting T_1 effects, will be sensitive to both the size of the exchanging lactate pool and the rate of entry of ^{13}C into the lactate pool. The sizes of the lactate and alanine pools, in turn, are sensitive to the [pyruvate] because of the high activity of lactate dehydrogenase and alanine aminotransferase in most tissues. Since the time course of a typical HP ^{13}C study is limited to about 120 seconds because of T_1 effects, it is not always clear whether the HP ^{13}C lactate (or alanine) signal has a contribution from changing pool size, exchange of isotope into the pool, or some combination. This information is essential for proper kinetic models designed to analyze HP ^{13}C data. The purpose of this study was to test whether pool sizes change significantly within 90 seconds after exposure to low concentrations of pyruvate in a highly metabolically active model.

Methods

Hearts were excised from anesthetized Sprague-Dawley rats (300-350 g) and perfused using standard Langendorff methods with Krebs-Henseleit medium. Three different combinations of oxidizable substrates were examined: 1) glucose (10 mM) alone, 2) glucose + 2 mM unlabeled pyruvate, or 3) glucose + 2 mM [3-$^{13}C_1$]-pyruvate. Seven groups were studied (n=2-3 in each group): *Group 1*, perfused with glucose alone for 30 min; *Groups 2 - 4*, perfused with glucose for 30 min and then switched to [3-$^{13}C_1$]-pyruvate for 30 seconds, 60 seconds or 90 seconds; *Groups 5 – 7,* perfused with glucose plus unlabeled 2 mM pyruvate for 30 min and then switched to 2 mM [3-$^{13}C_1$]-pyruvate for 30, 60 or 90 seconds. At the end of the perfusion period hearts were freeze-clamped. ^1H NMR spectra were obtained of the perchloric acid extracts (dissolved in ^2H_2O and spiked with an internal standard, 2,2-dimethyl-2-silapentane-5-sulfonic acid, DSS). The concentrations of ^{13}C and ^{12}C alanine and lactate were determined using Chenomx Software and analysis of the ^{13}C satellites in the ^1H NMR spectrum.

Results

After perfusion with glucose or unlabeled pyruvate the ^1H NMR spectrum showed the typical doublet in alanine and lactate methyl groups due to ^1H-^1H coupling. In the presence of [3-$^{13}C_1$]-pyruvate, the larger $^1J_{C\text{-}H}$ coupling was easily detected (upper panel). A schematic of the typical time course is shown to the right for experiment 1 (switching from glucose to glucose + [3-$^{13}C_1$]-pyruvate, and experiment 2 (switching from unlabeled pyruvate to [3-$^{13}C_1$]-pyruvate). In experiment 1, the size of both the lactate and alanine pools increased during continued perfusion between 30 and 90 seconds. Even after only 30 seconds, the ^{13}C fractional enrichment was relatively high, about 40%, indicating that exchange into the lactate and alanine pools is very rapid on the time-scale of a typical HP experiment. In experiment 2 (switching from unlabeled to labeled pyruvate at identical concentrations), the concentration of lactate and alanine was high even at 30 seconds, as expected, and there was no change in the concentration of either substrate. The fractional enrichment was low initially but rose to ~40% by 90 seconds for both lactate and alanine.



Panels from top to bottom: ^1H Spectra of lactate and alanine before and after perfusion with [3-$^{13}C_1$]-pyruvate, schematic of experimental design, concentration of total lactate and alanine, and fractional enrichment of lactate and alanine.

Conclusions

A typical *in vivo* study (bolus administration of HP-[1-$^{13}C_1$]-pyruvate) would be modeled by experiment 1, a rapid increase in the concentration of pyruvate at the tissue of interest. These results demonstrate that experiments involving a bolus injection of HP pyruvate should factor in time-dependent changes in pool size. An alternative design, experiment 2, maintains fixed pool sizes and the ^{13}C lactate or alanine signal is a consequence only of isotope exchange.