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In vivo investigation of cardiac metabolism in the rat using MRS of hyperpolarized [1-¹³C] and [2-¹³C]pyruvate

Sonal Josan^{1,2}, Jae Mo Park^{2,3}, Ralph Hurd⁴, Yi-Fen Yen², Adolf Pfefferbaum^{1,5}, Daniel Spielman^{2,3}, and Dirk Mayer^{1,2}

¹SRI International, Neuroscience Program, 333 Ravenswood Ave., Menlo Park, CA 94025

²Stanford University, Department of Radiology, Lucas MRI Center, 1201 Welch Rd. Stanford, CA 94305

³Stanford University, Department of Electrical Engineering, Stanford, CA 94305

⁴GE Healthcare Applied Sciences Laboratory, 333 Ravenswood Ave., Menlo Park, CA 94025

⁵Stanford University, Department of Psychiatry and Behavioral Sciences, 401 Quarry Rd., Stanford, CA 94305

Abstract

Hyperpolarized ¹³C MRS allows the in vivo assessment of pyruvate dehydrogenase complex (PDC) flux, which converts pyruvate to acetyl-coenzyme A (acetyl-CoA). [1-¹³C]pyruvate has been used to measure changes in cardiac PDC flux, with demonstrated increase in ¹³C-bicarbonate production after dichloroacetate (DCA) administration. With [1-¹³C]pyruvate, the ¹³C label is released as ¹³CO₂/¹³C-bicarbonate, and, hence, does not allow to follow the fate of acetyl-CoA. Pyruvate labeled in the C2 position has been used to track the ¹³C label into the TCA cycle and measure [5-¹³C]glutamate as well as study changes in [1-¹³C]acetylcarnitine with DCA and dobutamine. This work investigates changes in the metabolic fate of acetyl-CoA in response to metabolic interventions of DCA-induced increased PDC flux in the fed and fasted state, and increased cardiac workload with dobutamine in vivo in rat heart at two different pyruvate doses. DCA led to a modest increase in the ¹³C labeling of [5-¹³C]glutamate, and a considerable increase in [1-¹³C]acetylcarnitine and [1,3-¹³C]acetoacetate peaks. Dobutamine resulted in an increased labeling of [2-¹³C]lactate, [2-¹³C]alanine and [5-¹³C]glutamate. The change in glutamate with dobutamine was observed using a high pyruvate dose but not with a low dose. The relative changes in the different metabolic products provide information about the relationship between PDC-mediated oxidation of pyruvate and its subsequent incorporation into TCA cycle compared to other metabolic pathways. Using a high dose of pyruvate may provide an improved ability to observe changes in glutamate.

Keywords

hyperpolarized ¹³C; heart; metabolism; pyruvate; dose; dichloroacetate; dobutamine

Introduction

Hyperpolarized ¹³C magnetic resonance spectroscopy (MRS) has shown great potential for the in vivo assessment of metabolism, with several studies demonstrating applications to

cancer [1,2 (and references therein)] and investigation of cardiac metabolism [3–20]. The ability to probe a range of metabolic pathways with ^{13}C labeled tracers is a promising approach to evaluate changes in cardiac metabolism that are a characteristic of a number of diseases including diabetes, ischemia, hypertrophy and heart failure [5,6,21].

Hyperpolarized $[1-^{13}\text{C}]$ pyruvate (Pyr) has been used to assess flux through pyruvate dehydrogenase complex (PDC), which converts pyruvate to acetyl-coenzyme A (acetyl-CoA) in the mitochondria. In the process, the ^{13}C label is released as $^{13}\text{CO}_2$, which is in equilibrium with ^{13}C -bicarbonate (Bic) and provides a measure of PDC flux [7]. Previous reports have demonstrated a 2.6 to 3.3-fold increase in bicarbonate in response to increased cardiac PDC flux mediated by dichloroacetate (DCA) [12,13]. Golman et. al. also showed a decrease in bicarbonate signal with induced myocardial ischemia compared to normal myocardium [3]. However, reduced PDC flux measured via bicarbonate may not reflect lower TCA cycle activity, and using $[1-^{13}\text{C}]$ pyruvate does not allow the measurement of the fate of acetyl-CoA into the downstream metabolic steps. Hyperpolarized $[2-^{13}\text{C}]$ pyruvate and $[1,2-^{13}\text{C}]$ pyruvate have been used to follow the ^{13}C label into $[5-^{13}\text{C}]$ glutamate (Glu), generated from α -ketoglutarate in the tricarboxylic acid (TCA) cycle, as well as into $[1-^{13}\text{C}]$ acetylcarnitine (ALCAR) generated from acetyl-CoA via carnitine acetyltransferase (CAT) [14–17]. Schroeder et. al. [16] also showed changes in acetylcarnitine in response to the metabolic manipulations of increased PDC flux with DCA and increased cardiac workload with dobutamine (DOB). However, that study, which used a 0.25 mmol/kg dose of $[2-^{13}\text{C}]$ pyruvate, did not observe any changes in glutamate for either dichloroacetate or dobutamine. This work investigates whether interrogating cardiac metabolism using a higher pyruvate dose may permit the ability to observe changes in TCA cycle activity as reflected by ^{13}C labeling of glutamate.

Moreno et. al. [18] demonstrated in a Langendorff model of rat heart that substrate concentration can have a substantial impact on metabolic response in cardiac studies using hyperpolarized ^{13}C pyruvate. In their study, the fraction of acetyl-CoA derived from pyruvate oxidation increased from approximately 10% at the near-physiological pyruvate concentration of 0.12 mM to about 45% at 1 mM pyruvate and about 80% at 3 mM pyruvate. Further, the nutritional state of the heart also affected substrate utilization, with PDC flux at 6-mM pyruvate in the fasted state being roughly equal to that at 1-mM pyruvate in the fed state, as other sources of acetyl-CoA, such as fatty acids and ketones, compete with pyruvate for the production of acetyl-CoA. Thus, with greater pyruvate oxidation relative to fatty acids and ketones at high pyruvate concentrations, probing cardiac metabolism using a higher pyruvate dose may provide additional information about the ^{13}C label incorporation into different metabolic pathways.

In this study, ^{13}C MRS of hyperpolarized pyruvate was used to follow the ^{13}C label into lactate (Lac) and alanine (Ala) in the cytosol as well as through PDC to glutamate, acetylcarnitine and acetoacetate (Aca) using $[2-^{13}\text{C}]$ pyruvate in vivo in rat heart. The changes in these metabolic products were investigated in response to metabolic interventions such as increased PDC flux with dichloroacetate in the fed and fasted state, and to increased cardiac workload with dobutamine. The dependence on substrate dose of the metabolic response to dobutamine was also investigated using a “low” and a “high” dose of pyruvate. Experiments were also performed with hyperpolarized $[1-^{13}\text{C}]$ pyruvate to compare the DCA-induced change in bicarbonate with that in glutamate. These changes provide information about the relationship between PDC-mediated oxidation of pyruvate and its subsequent incorporation into TCA cycle relative to other metabolic pathways.

Methods

The polarized sample consisted of 30 μL of a mixture of 14-M (either $[1-^{13}\text{C}]$ or $[2-^{13}\text{C}]$) pyruvic acid and 15-mM Ox063 trityl radical, to which 3.5 μL of a 1:50 dilution of Dotarem (Guerbet, France) were added prior to polarization. The samples were polarized using a HyperSense system (Oxford Instruments Molecular Biotools, Oxford, UK) to achieve approximately 25% liquid-state polarization at dissolution. The polarized sample was dissolved with a solution of 80 mM NaOH, 40 mM Tris buffer, 0.1 g/L EDTA- Na_2 and 50 mM NaCl, leading to an 80-mM solution of hyperpolarized pyruvate with a pH of 7.4–7.5.

Animal Model

Healthy male Wistar rats (300–400 g body weight, $n=20$) were injected with approximately 3.2 – 4.2 mL (target dose = 1 mmol/kg body weight, unless stated otherwise) of the hyperpolarized pyruvate solution through a tail vein catheter at a rate of 0.25 mL/s. The rats were anesthetized with 1–3% isoflurane in oxygen (1.5 L/min) and respiration, heart rate and rectal temperature were monitored throughout the experiments with temperature regulated using a warm water blanket placed underneath the animals. All animal procedures were approved by the local Institutional Animal Care and Use Committee.

Each animal received two injections of the hyperpolarized pyruvate solution approximately 1.5 – 2 h apart, with either DCA or dobutamine infusion (details below) before the second pyruvate injection. Five experimental groups were used to assess cardiac metabolism. Group A ($n=3$) received $[1-^{13}\text{C}]$ pyruvate and DCA and provided control data for the change in bicarbonate due to DCA to compare with results in [12,13] given the differences in setup and acquisition parameters. Group B ($n=4$) received $[2-^{13}\text{C}]$ pyruvate and DCA. Similar to group B, rats in group C ($n=4$) received $[2-^{13}\text{C}]$ pyruvate and DCA, but were examined in the fasted state, with food withdrawn approximately 24 h prior to the experiment. Group D ($n=5$) received $[2-^{13}\text{C}]$ pyruvate and dobutamine before the second pyruvate injection. Similar to group D, group E ($n=4$) received $[2-^{13}\text{C}]$ pyruvate and dobutamine, but only received a 0.2 mmol/kg dose of pyruvate, with 1 ml injected volume, instead of 0.8 mmol/kg for group D. Groups D and E were used to examine the effects of pyruvate dose on the dobutamine-modulated changes in the metabolic pathways and also to compare to results in [16], which used a similar low pyruvate dose as group E. All rats except those in group C were allowed to feed freely prior to the experiment. The timing of the experimental sessions depended on scanner availability and most of the scans were performed in the afternoon, starting around 12 pm, with food removed at least 24 h prior to that for the fasted animals.

Dichloroacetate, a PDH kinase inhibitor, was used to stimulate flux through PDC [22]. The DCA solution (150 mg/kg body weight, dissolved in saline at 30 mg/mL) was injected into the tail vein similarly as in [12,13], with two thirds of the solution injected as a bolus and the remaining third injected slowly over 15 min. The second pyruvate injection was 15 min after end of the DCA infusion.

Dobutamine infusion was used to acutely increase cardiac workload. Dobutamine stimulates the β_1 adrenoceptors of the heart and increases heart rate and myocardial oxygen demand, and is also known to increase flux through PDC and the TCA cycle in rats in vivo [23]. Dobutamine (0.5 mg/kg body weight, dissolved in 1 mL saline) was injected into the tail vein over 10 minutes immediately prior to the second pyruvate injection. Heart rate increased during dobutamine infusion, reaching a plateau about 5 min into the infusion, and slowly returned to baseline in 10 to 20 min after the end of dobutamine infusion. With the pyruvate injection lasting about 12–16 s, and data acquired within 90 s of the pyruvate injection, the heart rate though somewhat variable during the acquisition remained higher than baseline, but slightly lower than the peak during dobutamine.

MR protocol

All experiments were performed on a clinical 3T MR scanner (GE Healthcare, Waukesha, WI) using custom-built transmit/receive ^{13}C and ^1H coils. The ^{13}C coil was a single-loop surface coil (inner diameter=28 mm) placed on the chest over the heart with the rat in the supine position. A quadrature volume rat ^1H coil (diameter=70 mm) was used for anatomical localization and to confirm the position of the ^{13}C coil with respect to the heart. A non-selective pulse-and-acquire sequence with an excitation flip angle of 6° , spectral width of 10 kHz and 4096 points was used to acquire ^{13}C spectra from the heart every 3 s over a 4-min period starting at the same time as the pyruvate injection. A 24- μs hard RF pulse was used to achieve a large excitation bandwidth to cover the wide chemical shift of the ^{13}C resonances. The time from dissolution to start of injection was 18 ± 1 s.

The MRS data were apodized with 15-Hz Gaussian line broadening and zero-filled by a factor of two. The metabolite time courses were calculated by integrating the peaks in the individual spectra in absorption mode. Metabolite levels were measured after summing up the absorption mode spectra from time 6 s to 90 s (i.e. frames 3 – 30) after $[1-^{13}\text{C}]$ pyruvate injection and 6 s to 72 s after $[2-^{13}\text{C}]$ pyruvate injection. The time windows for summation of the spectra were chosen based on the time-courses of the metabolic products to exclude the later time frames with little remaining signal. The slightly longer time window for $[1-^{13}\text{C}]$ pyruvate was based on the slightly longer lasting bicarbonate signal compared to the metabolic products from $[2-^{13}\text{C}]$ pyruvate. For the spectra displayed in Fig. 1, a zero-order and first-order phase correction was performed and the baseline was subtracted by fitting a spline to the signal-free regions of the spectrum.

The statistical analyses regarding the effect of DCA and dobutamine were performed on the ratios of metabolite to substrate levels calculated from the sum spectra. A two-tailed, paired t-test was used to compare metabolite-to-substrate ratios at injections 1 and 2, whereas an unpaired t-test was done when evaluating the ratios across groups.

Results

Representative ^{13}C spectra in Fig. 1 show the metabolic products observed after $[2-^{13}\text{C}]$ pyruvate (207.8 ppm) injection. The $[2-^{13}\text{C}]$ lactate (71 ppm) and $[2-^{13}\text{C}]$ alanine (53 ppm) resonances, produced from pyruvate via lactate dehydrogenase and alanine aminotransferase respectively, were observed as doublets due to ^{13}C - ^1H coupling. The ^{13}C label incorporation into the TCA cycle (via PDC and acetyl-CoA) was observed indirectly through $[5-^{13}\text{C}]$ glutamate (183.8 ppm) which is in isotopic exchange with the TCA cycle intermediate α -ketoglutarate via the oxoglutarate/malate carrier [24,25]. The metabolic products $[1-^{13}\text{C}]$ acetylcarnitine at 175.2 ppm, and acetoacetate with the ^{13}C label in the C_1 position at 177.3 ppm and the C_3 position at 212.7 ppm were also observed. Acetyl-CoA is reversibly converted to acetylcarnitine via carnitine acetyltransferase in the heart mitochondria and may act as a buffer for acetyl-CoA [16]. Acetoacetate is a ketone body formed using two molecules of acetyl-CoA resulting in ^{13}C labels in the C_1 and C_3 positions.

Figure 2 plots the time-courses averaged over 4 rats each for groups B and D, showing the effects of DCA and dobutamine infusions on the metabolic products. The error bars represent standard error. The spectra from each rat were individually normalized to the time-averaged pyruvate signal before averaging. The time-course of acetylcarnitine appears to be delayed post-DCA and post-dobutamine relative to the baseline time-course. However, a higher temporal resolution than the 3 s used here is needed to accurately quantify changes in timing. The baseline signal level of acetoacetate is slightly lower for group D compared to group B as the group D rats received a slightly lower pyruvate dose (approximately 0.8

mmol/kg instead of 1 mmol/kg due to their greater body weight ¹). The ratios of metabolic products to pyruvate for all groups are given in Table 1.

DCA

A comparison of the spectra acquired at baseline and post-DCA in Fig. 1 demonstrates the effect of the increased PDC flux on cardiac metabolism. With DCA administration, an increase was observed in glutamate, acetylcarnitine and acetoacetate labeling, while no changes were observed in either lactate or alanine. The large increase in both acetylcarnitine and acetoacetate peaks may indicate a route for disposal of excess acetyl-CoA being generated from the significantly increased PDC flux.

Representative ¹³C spectra from a fasted animal, acquired at baseline and post-DCA administration, are shown in Fig. 1. After 24 h fasting, the baseline ¹³C peaks of glutamate and acetylcarnitine were considerably lower than those of the free-fed animals, consistent with the reduced ¹³C bicarbonate production from [1-¹³C]pyruvate with overnight fasting in [8]. The increase with DCA for this group was much greater than for the free-fed group, leading to post-DCA metabolite ratios being similar in both groups. As the baseline acetoacetate levels of both groups were barely at the detection limit, it could not be evaluated whether the ¹³C labeling of acetoacetate was changed in the fasted group compared to the free-fed group.

Comparing the metabolite ratios before and after DCA, for group A injected with [1-¹³C]pyruvate, the average Bic/Pyr ratio increased from 0.031±0.001 at baseline to 0.086±0.002 post-DCA (mean±std, p<0.0009). Thus, the ratio increased to 2.81±0.13 relative to baseline, which is similar to previously reported values [12,13]. The ratios of metabolic products to pyruvate for all rats from groups B and C are given in Table 1 and plotted in Fig. 3. For the free-feeding rats in group B, the average ratios of Glu/Pyr, Alcar/Pyr and Aca/Pyr all increased from baseline to post-DCA (p<0.003 for Glu/Pyr, p<0.018 for Alcar/Pyr and p<0.019 for Aca/Pyr). Thus, the average change relative to baseline was 30±18% for glutamate, 78±36% for acetylcarnitine and about 10-fold for acetoacetate. However, the acetoacetate peak at baseline was barely at the detection threshold, making it hard to accurately quantify the change. The ¹³C₃ resonance of acetoacetate at 212.7 ppm partially overlapped with [2-¹³C]Pyr and the ¹³C₁ resonance was used to quantify the peak area and calculate ratios.

For the fasted rats in group C, the average ratios of Glu/Pyr, Alcar/Pyr and Aca/Pyr also increased from baseline to post-DCA (p<0.001 for Glu/Pyr, p<0.003 for Alcar/Pyr and p<0.004 for Aca/Pyr). Additionally, the baseline Glu/Pyr and Alcar/Pyr ratios of groups B and C differed significantly (unpaired t-test, p<0.001 for Glu/Pyr and p<0.001 for Alcar/Pyr).

The Lac/Pyr ratios (Table 1) showed a slight decrease with DCA that was statistically significant for group C (p<0.020), but not for group B (p=0.124). For both groups B and C, the Ala/Pyr ratios (Table 1) remained unchanged from baseline to post-DCA (p=0.956 for group B, and p=0.498 for group C).

Dobutamine

Representative ¹³C spectra for the [2-¹³C]pyruvate experiments, acquired at baseline and post-dobutamine administration (Fig. 1), demonstrate the effect of increased cardiac

¹The experiment sessions for groups D and E were performed a few weeks after those for A, B and C, resulting in somewhat greater body weight of those rats (336±20 g for groups A,B,C combined and 383±16 g for groups D,E) and hence slightly lower dose for group D (0.8 mmol/kg) compared to groups A, B and C (1 mmol/kg).

workload on the metabolic products. An increase was observed in $[2-^{13}\text{C}]$ lactate, $[2-^{13}\text{C}]$ alanine and in $[5-^{13}\text{C}]$ glutamate labeling with the higher TCA cycle activity from the elevated cardiac workload. Acetoacetate was at or below noise level in these cases, similar to the pre-DCA data above and did not change with dobutamine. Peak 6 in Fig. 1 is tentatively assigned to $[1-^{13}\text{C}]$ citrate (180.2 ppm), but was not reliably observed with sufficient SNR in all animals. Additionally, it could also have contributions from the nearby resonances of pyruvate-hydrate and alanine due to the 1% natural abundance $[1,2-^{13}\text{C}_1]$ pyruvate in the injected solution.

The glutamate peak overlaps with the upfield peak of the $^{13}\text{C}_1$ -lactate doublet produced from naturally abundant $[1,2-^{13}\text{C}_1]$ pyruvate in the hyperpolarized $[2-^{13}\text{C}_1]$ pyruvate sample. With dobutamine, lactate changed considerably and this overlap could potentially cause an overestimation of the change in glutamate. Therefore, for more accurate quantification, the lactate signal estimated from the downfield peak of $^{13}\text{C}_1$ -lactate doublet (185.8 ppm) was subtracted from the glutamate signal, similar to [15]. The downfield peak of the doublet is labeled in Fig. 1c as peak 7.

The ratios of metabolic products to pyruvate for group D are given in Table 1 and plotted in Fig. 3. The average Glu/Pyr ratio for this group increased from baseline to post-dobutamine ($p < 0.026$). The change in ratios between the two injections was also significant for Lac/Pyr ($p < 0.001$) and Ala/Pyr ($p < 0.017$). The effect on acetylcarnitine, though showing a trend to increase, was not significant given the large inter-subject variability in the response to dobutamine ($p = 0.278$).

The results of group E (Table 1 and Fig. 3), injected with a lower pyruvate dose, illustrate the effect of pyruvate dose on changes in the different metabolic pathways. In contrast to the increased glutamate signal observed for group D, and similar to that reported in [16], group E showed no significant difference in Glu/Pyr ratios with dobutamine infusion ($p = 0.609$). The change in acetylcarnitine for this group also did not reach significance ($p = 0.834$), though the animals show a similar trend as the results in [16] with 3 of the 4 animals showing a decrease. The increase in Lac/Pyr ($p < 0.003$) and Ala/Pyr ($p < 0.013$) for group E was significant and the percentage changes between the two injections were similar as those for the higher Pyr dose (Lac: $131 \pm 45\%$ for group E vs. $109 \pm 38\%$ for group D; Ala: $97 \pm 60\%$ for group E vs. $78 \pm 49\%$ for group D).

Discussion

Similar to Schroeder et. al. [16], acetylcarnitine increased with DCA, though the change observed here was greater (78% vs. 35%). In contrast, Schroeder et. al. did not observe a change in glutamate and did not report detecting acetoacetate. These differences could be due to the higher pyruvate dose used in this study, i.e. 1 mmol/kg here vs. 0.25 mmol/kg in [16]. Further, similar to [16] there was no change in glutamate signal with dobutamine for the low pyruvate dose (group E) here, while an increase in glutamate was observed using a high pyruvate dose (group D). This dose dependence of the results provides an insight into hyperpolarized ^{13}C MRS as a tool to interrogate metabolism in response to the substrate bolus injection, and the data may not reflect equilibrium metabolism given the supraphysiological doses used. Using a high dose of pyruvate may provide the improved ability to observe changes in glutamate, and thus probe TCA cycle activity, with metabolic perturbations in cardiovascular diseases.

Additionally, small differences in the dobutamine and DCA administration protocol could potentially also contribute to some of the dissimilarities in our results as compared to [16]. For instance, though the total dobutamine dose was the same, the dobutamine infusion

duration was 10 min here, with the elevated heart rate maintained throughout, as compared to 5 min in [16]. The post-DCA scan was performed 15 min after DCA here vs. immediately after infusion in [16].

The increase in [2-¹³C]lactate and [2-¹³C]alanine with dobutamine here is comparable with the rise in [1-¹³C]lactate and [1-¹³C]alanine observed by [20] with hyperpolarized [1-¹³C]pyruvate after dobutamine in pig hearts in vivo and with the increased lactate and alanine tissue levels after dobutamine in [26] with [2-¹³C]acetate infusion in canine hearts. Increased lactate was also observed in rat hearts perfused with radiolabeled glucose [27]. The lack of change in glutamate with dobutamine for the low dose group here and in [16] is also consistent with the unaltered glutamate concentration at elevated cardiac workload with radiolabeled glucose infusion in [23,27], while other reports have noted a decrease in glutamate labeling with [2-¹³C]acetate infusion [26,28] after dobutamine, potentially due to competition for α -ketoglutarate between the TCA cycle and the α -ketoglutarate – malate transport as part of the malate-aspartate shuttle [26].

In this study, the changes in PDC flux as measured by bicarbonate and in TCA cycle reflected indirectly via glutamate were assessed separately using [1-¹³C]pyruvate and [2-¹³C]pyruvate respectively. Though hyperpolarized [1,2-¹³C]pyruvate [15] or co-polarization of [1-¹³C]pyruvate and [2-¹³C]pyruvate [29] could allow simultaneous measurement of changes in bicarbonate and glutamate, the acetylcarnitine and acetoacetate peaks would likely be obscured by the much larger ¹³C₁ resonances of pyruvate at 173 ppm and alanine at 178 ppm. Glutamate also would at least partially overlap with the ¹³C₁ peak of lactate (which would be a doublet for [1,2-¹³C]pyruvate) at 185 ppm, making quantification difficult.

The MRS signal localization was achieved by the ¹³C surface coil placed on the chest over the rat heart. This could allow some signal contamination from surrounding tissue like skeletal muscle and liver and, especially for the injected substrate, from blood. To evaluate the contribution of signal from outside the heart, free induction decay chemical shift imaging (FIDCSI) of [2-¹³C]pyruvate and its metabolic products was performed in one rat (details and results in online supplement). The metabolic images showed that the signal for the products was predominantly from the heart and contributions from surrounding tissue, in particular from the liver, could be neglected, as expected given the sensitivity profile of the surface coil and the high metabolic activity of cardiac tissue.

There could be some contribution from the chest muscle but is also expected to be small based on the CSI images shown in the supplement. While slice-selective 2D or volumetric CSI would allow better localization of the signal to the heart, the wide spectral dispersion of the resonances resulting from [2-¹³C]pyruvate and the low signal-to-noise ratio of the metabolic products makes CSI challenging.

The appearance of acetoacetate signal is somewhat surprising as the heart is not normally a ketogenic organ. Liver is known to be the main site of ketone body formation, producing acetoacetate from acetyl-CoA via acetoacetyl-CoA and β -hydroxy- β -methylglutaryl-CoA (HMG-CoA) as intermediate steps in ketogenesis. However, other tissues can also form small amounts of ketone bodies [30–32] through the following reactions:

These reactions involve the enzymes β -ketothiolase, and 3-oxo-acid CoA transferase, respectively. The heart has much lower HMG-CoA synthase activity compared to liver, but

has high levels of α -ketothiolase [30]. Thus, though ketone body production in the heart may be negligible under physiological conditions, the heart does have the capacity to form acetoacetate under certain conditions. The pyruvate dose in the injected bolus is much higher than physiological level, and the excess of acetyl-CoA produced from it after DCA infusion may alter the behavior from equilibrium metabolic state.

While the liver produces acetoacetate, however, based on the surface coil sensitivity profile and the CSI data, signal contributions directly from the liver can be neglected. Another alternative source of the acetoacetate signal could be from the blood after synthesis in the liver, as this study could not differentiate between signal from heart muscle or from blood. However, that seems unlikely given the transport steps involved in delivery from liver mitochondria to blood to heart and T_1 losses during that time. While tissue analysis for the metabolites could not be performed in this study to verify the acetoacetate peak assignment, the observation of both resonances of $[1,3-^{13}\text{C}]$ acetoacetate at 177 and 212 ppm and their similar increase with DCA strongly suggests it as a likely candidate for those signals.

Acetoacetate can be reduced by α -hydroxybutyrate dehydrogenase and NADH to yield α -hydroxybutyrate, another ketone body. However, the $[1-^{13}\text{C}]$ α -hydroxybutyrate peak at 183.2 ppm could not be resolved from glutamate. The resonance of $[3-^{13}\text{C}]$ α -hydroxybutyrate at 68.4 ppm overlaps with the upfield peak of the $[2-^{13}\text{C}]$ lactate doublet and hence was not observed either. As there was no obvious increase in the upfield peak of the lactate doublet relative to the downfield peak after DCA infusion, and the Lac/Pyr ratio decreased with DCA in most cases, the increase observed for glutamate is likely not due to α -hydroxybutyrate.

Supraphysiological pyruvate concentrations are typically used for in vivo studies with hyperpolarized ^{13}C to achieve sufficient SNR of the metabolic products and to allow saturation of the targeted enzyme reactions so that the products are limited by enzyme activities and not substrate concentration. The study by Hu et al [33] performed 4 injections of 2.5 mL each of hyperpolarized pyruvate within 15 min in rats obtaining consistent and reproducible MRS data. Yen et al [34] also stated that blood samples drawn before and after ^{13}C pyruvate injections in a dog study (using a dose similar to the high dose here) showed rapid pyruvate metabolism and no cumulative effects with injections at 1.5 h intervals.

This work demonstrates the feasibility of measuring changes in the metabolite signals in vivo using hyperpolarized ^{13}C MRS. The ability to observe changes in these metabolic pathways enables further investigation into their role in cardiovascular diseases and may provide information about myocardial viability [5]. A rigorous analysis of the underlying mechanisms of these metabolic changes is beyond the scope of this study. The hyperpolarized signal includes contributions from flux/exchange and pool size effects and these factors cannot be differentiated in this study.

Conclusion

This work demonstrates in vivo measurement of changes in cardiac metabolism with DCA and dobutamine using MRS of hyperpolarized ^{13}C -labeled pyruvate. DCA led to an increase in the ^{13}C labeling of $[5-^{13}\text{C}]$ glutamate, $[1-^{13}\text{C}]$ acetylcarnitine and $[1,3-^{13}\text{C}]$ acetoacetate peaks. Dobutamine resulted in an increased labeling of $[2-^{13}\text{C}]$ lactate, $[2-^{13}\text{C}]$ alanine and $[5-^{13}\text{C}]$ glutamate. The relatively smaller increase in ^{13}C labeling of glutamate as compared to bicarbonate with DCA provides information about the relationship between PDC-mediated oxidation of pyruvate and its subsequent incorporation into TCA cycle relative to other metabolic pathways as seen by the increase in the acetylcarnitine and acetoacetate

resonances. The ^{13}C label incorporation into glutamate was found to depend on pyruvate dose with a high dose of pyruvate demonstrating increased ^{13}C labeling of glutamate after dobutamine, while no change was observed with a low pyruvate dose. These experiments illustrate the importance of considering substrate dose in interpreting results and that while hyperpolarized ^{13}C MRS provides a unique tool to interrogate in vivo metabolic pathways, fluxes, and enzyme activities, the data may not correspond to baseline metabolism as substrate doses typically exceed normal equilibrium levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

CAT	carnitine acetyltransferase
CSI	chemical shift imaging
DCA	dichloroacetate
DOB	dobutamine
NADH	nicotinamide adenine dinucleotide, reduced form
PDH	pyruvate dehydrogenase
PDC	pyruvate dehydrogenase complex
SNR	signal-to-noise ratio
TCA	tricarboxylic acid

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Figure 1.

(A) Representative ^{13}C MRS spectra from rat heart in vivo showing the peaks observed after hyperpolarized $[2\text{-}^{13}\text{C}]\text{pyruvate}$ bolus injection, normalized to pyruvate signal. Pyr-H represents pyruvate-hydrate. The zoomed-in spectra for the dotted line region are shown in (B-D). An increase in glutamate (peak 2), acetoacetate (peaks 1 and 3) and acetylcarnitine (4) labeling was observed with increased PDH flux post-DCA for free-fed (B) and fasted (D) rats. Peak 5 is the natural abundance $^{13}\text{C}_1$ doublet peak from natural abundance $[1,2\text{-}^{13}\text{C}]\text{pyruvate}$ in the injected solution. The spectra in (c) show an increase in lactate, alanine and glutamate after dobutamine (DOB). Peak 6 (180.2 ppm) is tentatively assigned to citrate, but was not reliably observed with sufficient SNR in all animals and could include contributions from one of the peaks of $[1,2\text{-}^{13}\text{C}_1]\text{pyruvate-hydrate}$ doublet. Peak 7 is the downfield peak of $^{13}\text{C}_1\text{-lactate}$ doublet with the upfield peak overlapping with glutamate.

Figure 2.

Metabolite time-courses averaged over four rats from the DCA and dobutamine groups. The data acquisition started at the same time as the pyruvate injection. The post-DCA data was acquired 15 min after DCA infusion. The post-dobutamine data was acquired immediately after dobutamine infusion.

Figure 3.

Metabolite-to-pyruvate ratios from rat heart in vivo after hyperpolarized $[2-^{13}\text{C}]$ pyruvate injection at baseline and after DCA or dobutamine (DOB). The ratios show an increase in glutamate, acetylcarnitine and acetoacetate labeling with DCA for both free-fed and fasted animals. Lactate showed a decreasing trend post-DCA, which was statistically significant for the fasted group, but not for the free-fed group. Alanine did not change with DCA. The ratios show an increase in lactate, alanine and glutamate labeling with increased cardiac workload due to dobutamine for the high Pyr dose, but no significant change in glutamate for low Pyr dose. There was no statistically significant change in acetylcarnitine with dobutamine.

Table 1

Metabolite-to-substrate ratios expressed in % (e.g. 100*Glu/Pyr) from in vivo rat heart after a bolus injection of hyperpolarized [2-¹³C]pyruvate. Each animal received 2 pyruvate injections, with either DCA (group B and C) or Dobutamine (group D and E) administration in between. Group C was fasted for 24 h prior to the imaging experiment. Group E received a lower dose of pyruvate (0.2 mmol/kg) compared to group D (0.8 mmol/kg). The acetoacetate signal for groups D and E was not quantified due to low SNR and is not reported here.

	Group B	Group C	Group D	Group E
Metabolite-to-pyruvate ratios (%)	DCA	Fasted, DCA	Dobutamine	Dobutamine, low Pyr dose
Glutamate, pre	0.48 ± 0.04	0.13 ± 0.01	0.48 ± 0.17	1.14 ± 0.48
post	0.61 ± 0.05	0.51 ± 0.05	0.72 ± 0.09	1.24 ± 0.79
Acetylcarnitine, pre	1.21 ± 0.24	0.15 ± 0.09	1.46 ± 0.88	2.30 ± 1.36
post	2.11 ± 0.42	1.61 ± 0.38	1.87 ± 1.02	2.20 ± 1.75
Acetoacetate, pre	0.03 ± 0.02	0.03 ± 0.01	--	--
post	0.30 ± 0.12	0.36 ± 0.08	--	--
Lactate, pre	3.47 ± 0.08	3.19 ± 0.11	4.44 ± 0.89	7.60 ± 2.21
post	3.22 ± 0.23	2.80 ± 0.15	9.84 ± 1.67	17.05 ± 2.85
Alanine, pre	0.88 ± 0.36	0.62 ± 0.28	1.23 ± 0.15	1.24 ± 0.31
post	0.88 ± 0.37	0.67 ± 0.25	2.36 ± 0.66	2.35 ± 0.37

The bold text/shaded cells indicate statistically significant ($P < 0.05$) difference between pre- and post- values.