

# Imaging Regional Metabolic Changes in the Ischemic Rat Heart In Vivo Using Hyperpolarized [1-<sup>13</sup>C]Pyruvate

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**Abbreviations:** Magnetic resonance imaging (MRI), gadolinium (Gd), electrocardiogram (ECG), left anterior descending (LAD), magnetic resonance (MR), repetition time (TR), echo time (TE)

## ABSTRACT

We evaluated the use of hyperpolarized <sup>13</sup>C magnetic resonance imaging (MRI) in an open-chest rat model of myocardial infarction to image regional changes in myocardial metabolism. In total, 10 rats were examined before and after 30 minutes of occlusion of the left anterior descending coronary artery using hyperpolarized [1-<sup>13</sup>C]pyruvate. Cardiac metabolic images of [1-<sup>13</sup>C]pyruvate and its metabolites [1-<sup>13</sup>C]lactate, [1-<sup>13</sup>C]alanine, and [<sup>13</sup>C]bicarbonate were obtained before and after ischemia. Significant reduction in the [1-<sup>13</sup>C]alanine and [1-<sup>13</sup>C]lactate signals were observed in the ischemic region post ischemia. The severity of the ischemic insult was verified by increased blood levels of troponin I and by using late contrast-enhanced MRI that showed enhanced signal in the ischemic region. This study shows that hyperpolarized MRI can be used to image regional metabolic changes in the in vivo rat heart in an open-chest model of ischemia reperfusion. Hyperpolarized MRI enables new possibilities for evaluating changes in cardiac metabolism noninvasively and in real time, which potentially could be used for research to evaluate new treatments and metabolic interventions for myocardial ischemia and to apply knowledge to future application of the technique in humans.

## INTRODUCTION

Changes in myocardial metabolism are known to be one of the earliest markers of ischemic heart disease (1). Rodent animal models offer unique opportunities to study these changes. However, imaging the rat heart in vivo offers several challenges. In comparison to humans or pigs, the rat heart is much smaller (~20 mm in length) with a relatively thinner myocardium, and furthermore, it beats 5–6 times faster (up to 450 bpm). Despite this, some of the most used and best characterized models of cardiac diseases are developed in rats.

Hyperpolarized [1-<sup>13</sup>C]pyruvate magnetic resonance imaging (MRI), an emerging imaging technique, can assess and visualize regional metabolic changes in intact beating heart in real time (2–7). The advantage of hyperpolarization is that the magnetic resonance spectroscopy signal from <sup>13</sup>C-labeled metabolites can be increased >10,000-fold (8), making it possible to detect low concentrations of the metabolites in vivo and to create metabolic images of the signal. Moreover, hyperpolarized

MRI enables monitoring of several steps in metabolic pathways, adding information of fluxes through specific enzymes in the cardiac myocytes cytosol and mitochondria. It can further easily be used in combination with conventional proton MRI to assess cardiac anatomy, function, perfusion, and viability with gadolinium (Gd)-based contrast agents.

In this study, hyperpolarized [1-<sup>13</sup>C]pyruvate was used. Pyruvate is the end product of glycolysis and a key substrate for energy production through tricarboxylic acid cycle. After intravenous injection, hyperpolarized [1-<sup>13</sup>C]pyruvate is taken up by the myocytes and converted into [1-<sup>13</sup>C]lactate via the enzyme lactate dehydrogenase and [1-<sup>13</sup>C]alanine via alanine aminotransferase, both enzymes located in the cytosol. Furthermore, [1-<sup>13</sup>C]pyruvate is converted into acetyl coenzyme A via the pyruvate dehydrogenase enzyme complex in the mitochondrial membrane, and in the process, the <sup>13</sup>C-atom in the C-1 position of the pyruvate molecule is transferred to (13) CO<sub>2</sub> in equilibrium with the large bicarbonate pool via the enzyme carbonic

anhydrase. The production of [<sup>13</sup>C]bicarbonate reflects the mitochondrial status, whereas the production of [1-<sup>13</sup>C]lactate and [1-<sup>13</sup>C]alanine reflects the general metabolic state of the myocytes, according to the location and activity of the enzymes in the cell. The metabolic consequences of myocardial ischemia have previously been shown in animal models, primarily in isolated perfused rat hearts (2, 7, 9) or in pigs (3, 6, 10). Recently, the technique has been applied in the in vivo rat heart in a complicated closed survival model of acute myocardial ischemia (11). In the present paper, we show an alternative approach to image the metabolic effects of regional myocardial ischemia in the in vivo rat heart, and demonstrate how challenges connected with its use in the small rapidly beating heart can be circumvented.

## METHODS

### Animals

All experiments were approved by The Danish Animal Experiments Inspectorate (License number: 2007/561-1350). In total, 12 male Sprague-Dawley rats (Taconic Europe, Denmark) weighing 250–350 g were examined in this study; 10 were examined with conventional MRI and hyperpolarized [1-<sup>13</sup>C]pyruvate before and 2 hours after ischemia. The remaining 2 rats were sham-operated and used only for evaluation of troponin I blood levels. All rats were given water and standard rat chow ad libitum. The preischemic scan was performed 1 or 2 days before the ischemic insult was performed to reduce the time the rats were anesthetized, which potentially could affect our results. The preischemic scan was performed as described below but without the surgery. Rats were anesthetized with 4% isoflurane. The rats were intubated and connected to a small animal ventilator (SAR-830/P, IITC Life Science, CA). The ventilator was supplied with 1.75 L/min atmospheric air with additional 0.25 L/min oxygen mixed with 1.6%–2% isoflurane to maintain light anesthesia. Respiration was kept at 72 breaths/min. pCO<sub>2</sub> was monitored on a NPB-75MAX Capnograph (Nellcor Puritan Bennett Inc, USA) connected to the ventilator. A catheter was introduced into the tail vein for intravenous administration of the hyperpolarized [1-<sup>13</sup>C]pyruvate solution. Another catheter was introduced in the left femoral artery for blood collection. During scanning, the animals were placed on a heating pad, and temperature, electrocardiogram (ECG), and expiration gases were monitored (body temperature: 37.0–38.0°C, expiration CO<sub>2</sub>: 3.5–4.0 kPa).

### Ischemic Heart Model

Myocardial ischemia was induced by a previously described open-chest technique (12, 13). The rats were anesthetized, intubated, and connected to a small animal ventilator as described above. For additional pain relief, 0.05 μg/g buprenorphine (Temgesic, Reckitt Benckiser, Søborg, Denmark) was given subcutaneously 15–30 minutes before surgery. After a left thoracotomy and a pericardiectomy, the left anterior descending (LAD) artery was occluded by placing a ligature around a little plastic tube placed on top of the branch. Ischemia was verified visually by bleaching and blue-coloring of the myocardium distal to the ligature. The ligature was placed to achieve an ischemic area covering ~1/2 of the anterior wall of the left

ventricle including the apex. The LAD coronary artery was occluded for 30 minutes, resulting in severe ischemia and infarction. Ischemia was followed by reperfusion achieved by releasing the tension of the ligature. A clear change in color from blue to red confirmed reperfusion. If reperfusion was not achieved, the rats were excluded from the study. For evaluation of tissue damage by the cardiac-specific biomarker troponin I, blood samples were drawn before ischemia and 1 and 2 hours after reperfusion in 6 of the 10 ischemic rats (owing to problems with the blood clotting in the catheter in the remaining 4). The level of troponin I was analyzed on an AQT90 Flex (Radiometer, Denmark). The 2 sham-operated animals underwent the surgical procedure including placing the ligature, but without occluding the artery.

### Hyperpolarization

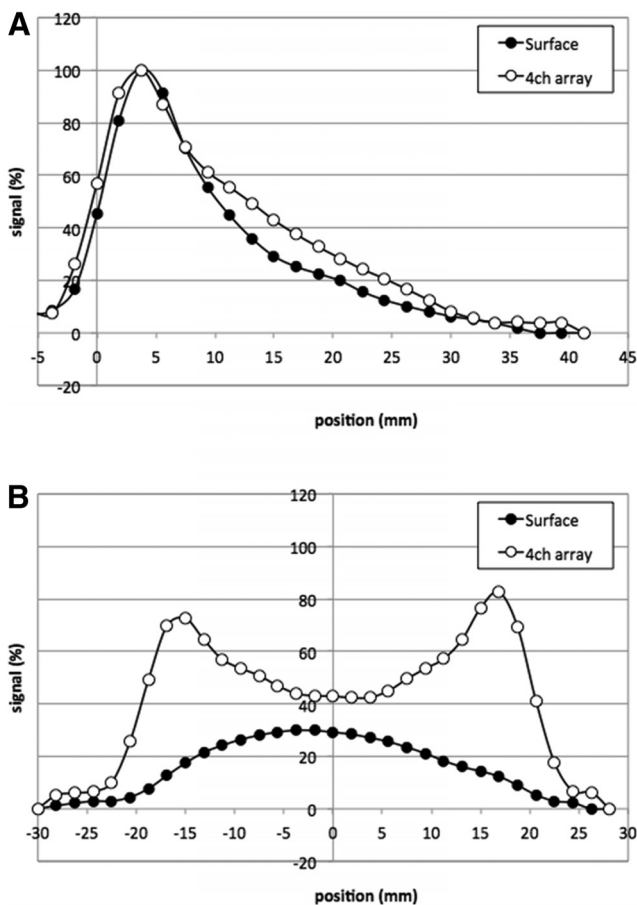
Here, 20 μL (~26 mg) of [1-<sup>13</sup>C]pyruvic acid (Sigma Aldrich, Germany) with 15mM trityl radical OX063 (Oxford Instruments, UK) and 1.5mM Dotarem (Guerbet, France) was loaded into a polarizer (HyperSense, Oxford Instruments, UK). The sample was dissolved in a neutralizing buffer (80 mM TRIS, 100 mg/L EDTA, 50 mM NaCl, 80mM NaOH), achieving a final concentration of 80 mM [1-<sup>13</sup>C]pyruvate (pH 7.0–8.0, temperature ~30°C, isotonic).

### MRI

A 4.7 T preclinical MRI and magnetic resonance spectroscopy system (Agilent, Santa Clara, CA) at the Danish Research Centre for Magnetic Resonance was used for the magnetic resonance (MR) experiments. The rats were placed supine in a <sup>13</sup>C/<sup>1</sup>H radiofrequency volume coil, and either a <sup>13</sup>C circular receive surface coil or a <sup>13</sup>C 4-channel receive array coil was placed over the heart (all coils from RAPID Biomedical GmbH, Germany). The volume coil had an inner diameter of 72 mm. The surface coil had a diameter of 20 mm, and it was sensitive to a depth of ~15 mm into the animal. The array coil consisted of 4 long elements of length 42.5 mm, each element parallel to each other covering the entire chest wall, and was sensitive to a depth of ~20 mm. The coil sensitivity profiles of the surface coil and the array coil in the transversal image plane are compared in Figure 1, A and B. B0 shimming was performed, and anatomical long-axis proton MR images were acquired for spatial localization of the heart using a cardiac- and respiratory-gated cine pulse sequence (repetition time [TR] = 195 milliseconds; echo time [TE] = 3 milliseconds; field of view = 60 × 120 mm<sup>2</sup>; section thickness = 2 mm; matrix size = 128 × 256, number of cardiac phases = 8). The position of the coils was verified by an external marker (oil pellet) placed on the top of the surface coil. On the array coil, the oil pellet was placed on one side of the coil and the animal was carefully placed so that the most sensitive part of the coil was closest to the heart.

### Hyperpolarized <sup>13</sup>C MRI

Via the tail vein catheter, 1.0 mL of isotonic hyperpolarized [1-<sup>13</sup>C]pyruvate solution was injected over 7–10 seconds; 7 s after the end of injection, an ECG and respiratory-gated section-selective chemical shift imaging sequence was started (section thickness = 5 mm, flip angle = 10°, circular spiral *k*-space 12 ×

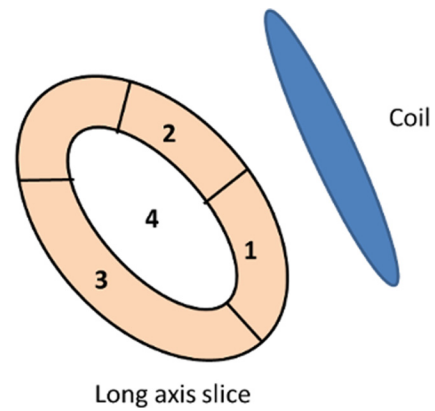


**Figure 1.** Coil profiles. Sensitivity profiles of the circular surface receive coil and the 4-channel array receive coil are compared in the axial image plane. The sensitivity was measured using a spherical phantom containing nonthermal polarized [<sup>13</sup>C]pyruvate. The signals are normalized to their maximum (100%). The coil sensitivity into the animal (from coil surface to the heart) (A) and the sensitivity length (along the coils in the axial image plane, in a distance from the coil where heart is assumed to be located) (B).

12 trajectory matrix, TR = 69 milliseconds, TE = 1.86 milliseconds, field of view = 25 × 25 mm<sup>2</sup>). The chemical shift imaging sequence was acquired from the same long-axis section through the heart as the one used for the proton cine imaging.

### Data Analysis

The [<sup>13</sup>C]pyruvate, [<sup>13</sup>C]lactate, [<sup>13</sup>C]alanine, and [<sup>13</sup>C]bicarbonate signals were quantified and mapped using in-house written MATLAB program (The MathWorks, Natick, MA). [<sup>13</sup>C]Pyruvate hydrate was also detected in the <sup>13</sup>C spectrum, but because it is not a direct metabolic product of pyruvate, it was not included in the quantification. The postprocessing quantification included apodization and zero-filling of the spatial dimensions to a matrix size of 32 × 32. The data were quantified in magnitude mode. Spectral analysis was performed by simple



**Figure 2.** Illustration of region of interest and coil placement. Schematic of the different regions used for the regions of interest analysis in a long-axis section through the myocardium: apex, anterior wall, posterior wall, and left ventricular lumen.

integration at predefined frequency offsets of the metabolites relative to the frequency of the pyruvate peak. The chemical shift imaging data were presented as metabolic maps, which were registered to the corresponding first cardiac-phase cine image (proton MRI).

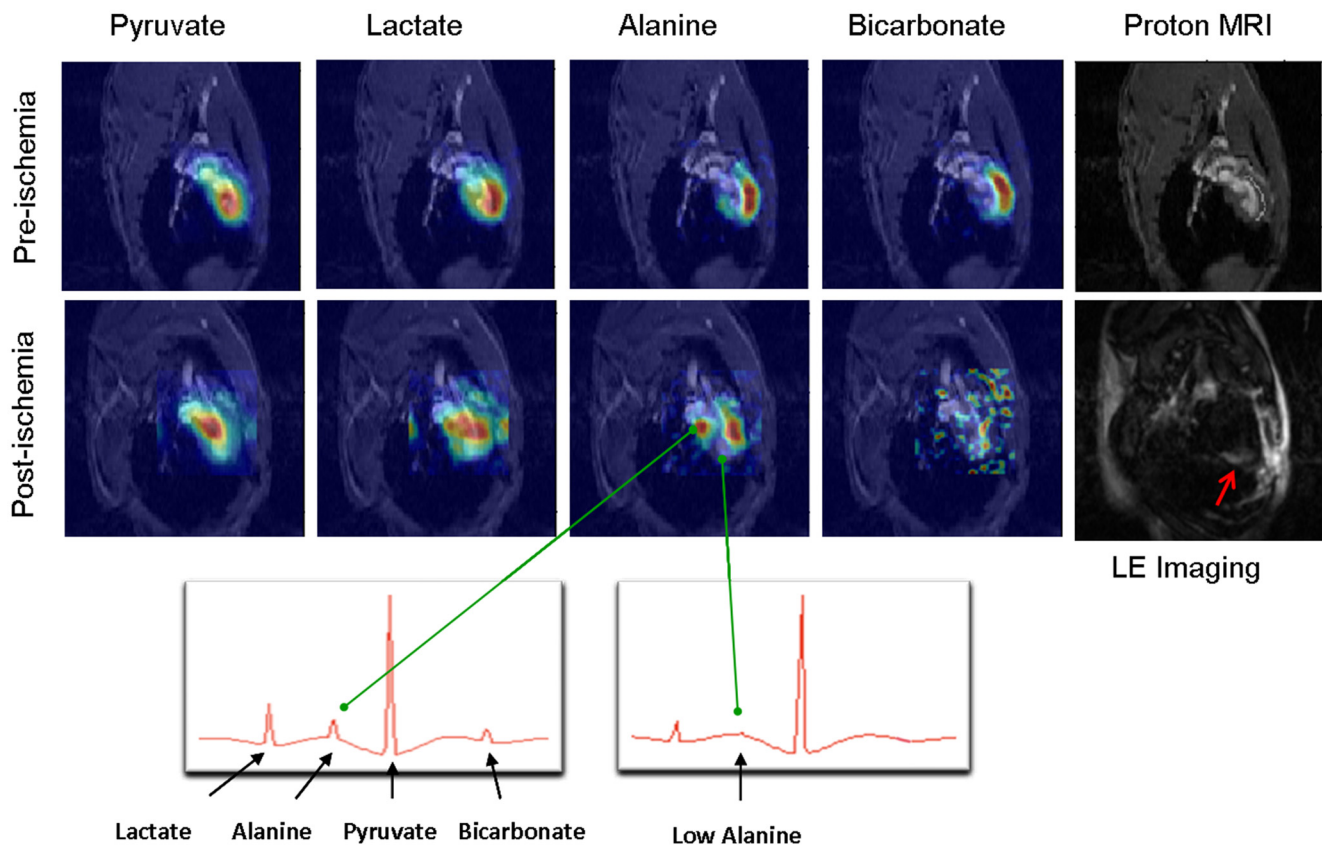
The effect of ischemia on the cardiac metabolism was quantified and illustrated using a region of interest analysis using the bullseye analogy (14). The myocardium was divided in 4 regions: apex (ischemic area in animals with lesion), anterior wall (close to the coil), posterior wall (remote from the coil), and left ventricular lumen (Figure 2). Hyperpolarized [<sup>13</sup>C]alanine, [<sup>13</sup>C]lactate, and [<sup>13</sup>C]bicarbonate measured in all 4 regions and normalized to (divided by) the [<sup>13</sup>C]pyruvate value for the corresponding compartment, were used for comparison between healthy (preischemia) and diseased (postischemia) hearts.

### Late Enhancement MRI

In 7 of the rats, the infarct area was also verified by comparing the metabolic maps visually with proton late Gd enhancement images. ECG-gated inversion recovery gradient-echo MR images were obtained 10–20 minutes after the injection of 0.3 mmol/kg Gd-based contrast agent (Dotarem, Guerbet). The inversion time was adjusted individually for each rat (350–500 milliseconds) to obtain the best contrast between the Gd-enhanced tissue and the surrounding healthy tissue (TR = 600 milliseconds; TE = 10 milliseconds; field of view = 60 × 120 mm<sup>2</sup>; section thickness = 2 mm; matrix size = 192 × 512).

### Statistics

A 2-way repeated ANOVA was performed to assess the differences between pre- and postischemic areas, and multiple comparisons were performed using Bonferroni post hoc correction. Myocardial damage marker troponin I was assessed by a paired *t* test assuming equal standard deviation, between baseline and 1 hour in the ischemia group (owing to unequal distribution of data points and insufficient sham animals). Normality was as-



**Figure 3.** Hyperpolarized <sup>13</sup>C metabolic images. Representative metabolic images of cardiac metabolism before ischemia (healthy heart, upper row) and after ischemia (30 minutes of ischemia followed by 2 hours of reperfusion, lower row) for pyruvate, lactate, alanine, and bicarbonate (field of view = 25 × 25 mm<sup>2</sup>, section thickness = 5 mm, flip angle = 10°). The last column to the right shows the proton magnetic resonance imaging (MRI) of the heart: upper row, before ischemic without enhancement; lower row, after ischemia with late enhancement image. In the late enhancement image, enhanced signal can be observed in the distal ischemic region of the anterior myocardial wall (red arrow) and in the tissue of the anterior chest wall.

essed with quantile–quantile plots. A value of  $P \leq .05$  was considered statistically significant. Statistical analysis was performed using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA).

## RESULTS

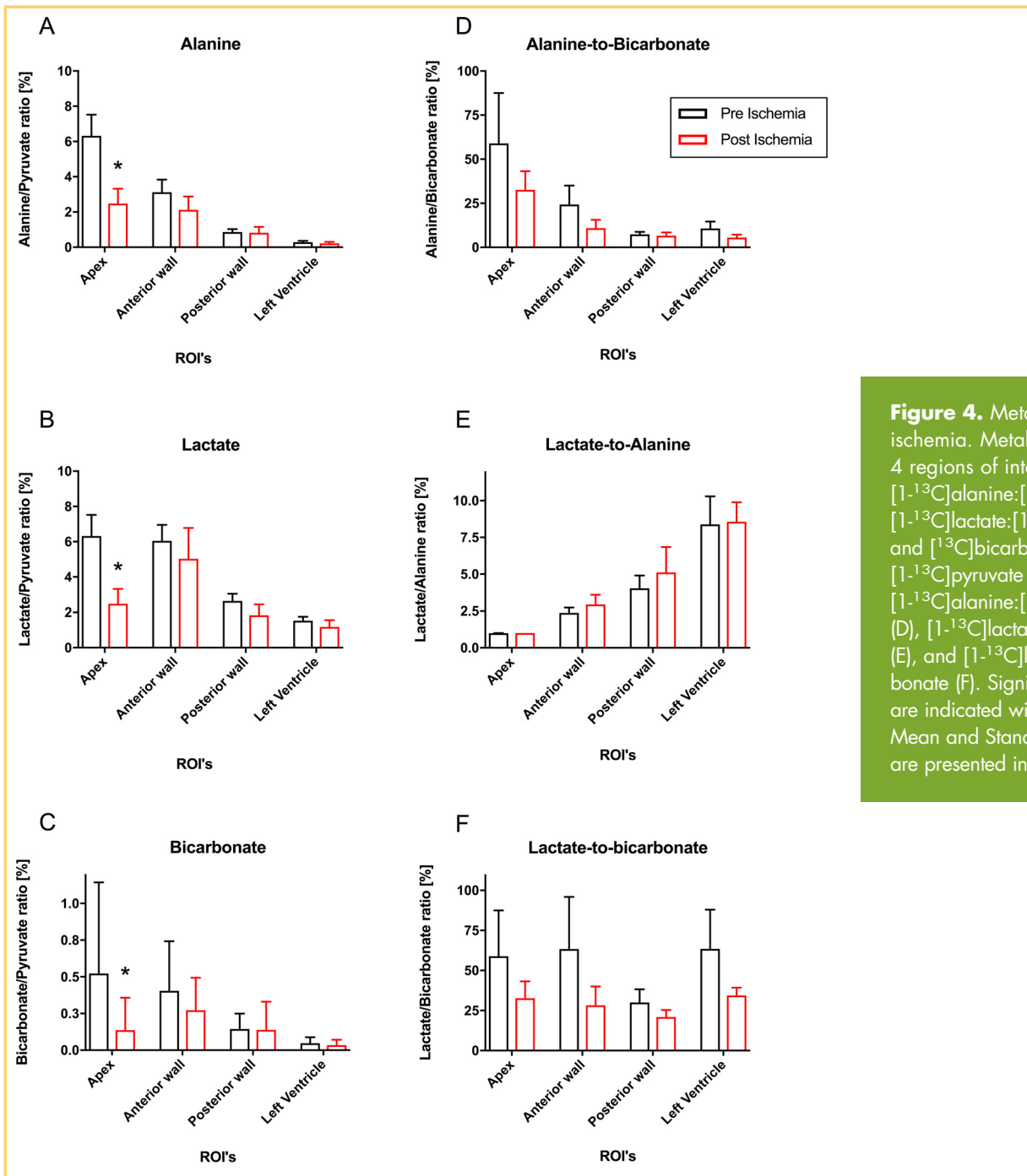
### Hyperpolarized <sup>13</sup>C MRI of the Healthy Heart (Preischemia)

Hyperpolarized signal from [<sup>1-<sup>13</sup>C</sup>]pyruvate, [<sup>1-<sup>13</sup>C</sup>]lactate, [<sup>1-<sup>13</sup>C</sup>]alanine, and [<sup>13</sup>C]bicarbonate was detected in the hearts of all animals before ischemia. As expected, signal from [<sup>1-<sup>13</sup>C</sup>]pyruvate was primarily detected from blood inside the ventricle (Figure 3, column 1). Signals from [<sup>1-<sup>13</sup>C</sup>]lactate, [<sup>1-<sup>13</sup>C</sup>]alanine, and [<sup>13</sup>C]bicarbonate were confined to the myocardium (Figure 3, columns 2–4). Because of the coil sensitivity profile, signals were primarily visual in the anterior wall of the myocardium closest to the coil. In some cases, lactate signal was observed in the injured chest wall and in the blood inside the ventricle, presumably because of the operative procedure (Figure 3, column 2).

### Effects of Ischemia

From the metabolic maps, we can observe that [<sup>1-<sup>13</sup>C</sup>]pyruvate almost exclusively is seen in the ventricle and [<sup>1-<sup>13</sup>C</sup>]lactate is seen both in the ventricle and the myocardium, whereas the [<sup>1-<sup>13</sup>C</sup>]alanine and [<sup>1-<sup>13</sup>C</sup>]bicarbonate are seen exclusively in the myocardium.

Apparent changes are seen in the raw lactate, alanine, and bicarbonate images before and after ischemia (Figure 3, columns 2–4). No apparent differences are observed in the pyruvate images (Figure 3, column 1). A decrease in the [<sup>1-<sup>13</sup>C</sup>]alanine signal is observed in the ischemic region (distal, apex region) of the anterior wall after ischemia compared with before ischemia (Figure 3, column 3). An overall lower [<sup>1-<sup>13</sup>C</sup>]bicarbonate signal is observed after ischemia. A statistical significant effect of ischemia was observed in all the metabolite-to-pyruvate ratios when comparing the interaction between time (before and after ischemia) and intraregional differences (regions of interest). The multiple comparison test showed a statistical significant difference between before and after ischemia, specifically in the apex for [<sup>1-<sup>13</sup>C</sup>]alanine:[<sup>1-<sup>13</sup>C</sup>]pyruvate ( $P < .0001$ ); [<sup>1-<sup>13</sup>C</sup>]lactate:



**Figure 4.** Metabolic effect of ischemia. Metabolic ratios in the 4 regions of interest. Left: [<sup>1-13</sup>C]alanine:[<sup>1-13</sup>C]pyruvate (A), [<sup>1-13</sup>C]lactate:[<sup>1-13</sup>C]pyruvate (B), and [<sup>13</sup>C]bicarbonate:[<sup>1-13</sup>C]pyruvate (C). Right: [<sup>1-13</sup>C]alanine:[<sup>13</sup>C]bicarbonate (D), [<sup>1-13</sup>C]lactate:[<sup>1-13</sup>C]alanine (E), and [<sup>1-13</sup>C]lactate:[<sup>13</sup>C]bicarbonate (F). Significant differences are indicated with an asterisk. Mean and Standard Error of Mean are presented in the figure.

[<sup>1-13</sup>C]pyruvate ( $P = .0001$ ); and the [<sup>1-13</sup>C]bicarbonate:[<sup>1-13</sup>C]pyruvate ( $P = .002$ ) (Figure 4A–C). Comparing the individual metabolites against each other is interesting because it could indicate a shift from one metabolic pathway to another. However, no effect of ischemia could be observed in the [<sup>1-13</sup>C]alanine:[<sup>1-13</sup>C]bicarbonate, [<sup>1-13</sup>C]lactate:[<sup>1-13</sup>C]alanine, or the [<sup>1-13</sup>C]lactate:[<sup>1-13</sup>C]bicarbonate signals (Figure 4D–F).

### Severity of Ischemia

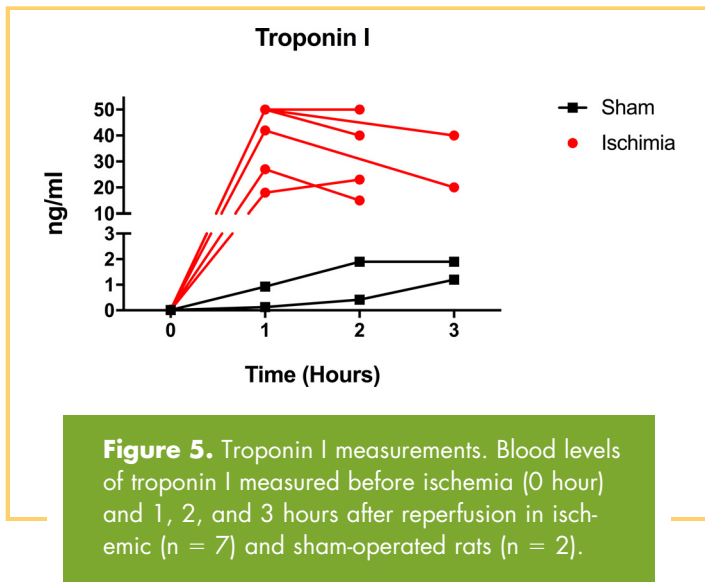
Elevated signal in the late-enhancement images corresponded to the area, which showed reduced <sup>13</sup>C signal in the [<sup>1-13</sup>C]alanine maps (Figure 3, column 5). The troponin I blood level peaked 1 hour after reperfusion ( $41.0 \pm 13.2$  ng/mL, mean  $\pm$  standard deviation,  $P = .0002$ ) and remained elevated 2–3 hours after

reperfusion (Figure 5). In the 2 sham-operated rats, the 1-hour value was in the normal range, 0.13 and 0.93 ng/mL. Further, as mentioned in the Methods, ischemia was verified visually by observing bleaching and blue coloring of the myocardium distal to the ligature, and reperfusion was confirmed by a change in color of the myocardium from blue to red.

## DISCUSSION

### Main Finding

The present study shows how hyperpolarized MRI can be used to image regional metabolic changes in the in vivo rat heart following 30 minutes of ischemia. The observed decrease in [<sup>1-13</sup>C]alanine, [<sup>1-13</sup>C]bicarbonate, and [<sup>1-13</sup>C]lactate signal in the reperfused ischemic area likely reflects an overall depression of



**Figure 5.** Troponin I measurements. Blood levels of troponin I measured before ischemia (0 hour) and 1, 2, and 3 hours after reperfusion in ischemic (n = 7) and sham-operated rats (n = 2).

the cellular metabolism following 30 minutes of severe ischemia and cell damage due to low supply of oxygen and nutrients to the area. The metabolic maps show that changes in [<sup>1-13</sup>C]alanine signal are a good marker for regional cellular damage because the signal is well confined to the myocardium. There is less disturbing contribution of [<sup>1-13</sup>C]alanine from the blood or surrounding tissue, which can be the case for [<sup>1-13</sup>C]lactate, which is produced in the injured tissue and transported out of the cells into the blood stream. Lactate dehydrogenase has previously been used as a serum biomarker of myocardial infarction in the clinic, so enzyme leak may also contribute to the reduced regional lactate and alanine signal (15). The severity of the ischemic insult is supported by observed increase in blood levels of troponin I, which is released to the blood because of myocardial damage (16), as well as the late enhanced Gd signal observed specifically in the ischemic region.

Our results support the recent findings by Oh-Ici, et al. (11). In this study ischemia was induced by a closed chest technique in rats. The LAD was occluded for 15 minutes and the [<sup>1-13</sup>C]pyruvate metabolism evaluated after 3 minutes, 30 minutes, 1 hour and 1 week respectively. In the current study, we used an open-chest model, and the effect of 30 minutes of ischemia was evaluated 2 hours after reperfusion. Interestingly the study by Oh-Ici, et al. shows an immediate change following ischemia by a tendency toward a higher mean lactate:bicarbonate ratio from 3 minutes to 1 week later. Higher mean lactate:bicarbonate ratio indicates alterations in the balance between the aerobic and anaerobic glycolysis and thus deviates from the findings in the present study. We speculate that this discrepancy might originate from the difference in induction and duration of ischemia (15 minutes vs 30 minutes in our study), as a doubling time of a total acute ischemia rat heart without collaterals is quite substantial and expected to result in more severe metabolic changes. However, both studies show that hyperpolarized [<sup>1-13</sup>C]pyruvate is useful for the evaluation of metabolic changes following cardiac ischemia in rats.

### Perfusion

As mentioned above, perfusion may be an interesting confounding factor to the metabolic changes we observe. Low perfusion to the ischemic area would result in lower/slower delivery of [<sup>1-13</sup>C]pyruvate to the ischemic area, which would result in a lower metabolic conversion of pyruvate to its metabolic products as we observe. In the present study, reperfusion was ensured visually in all rats before scanning. Rats in whom reperfusion was not observed were immediately excluded from the study. This is an advantage over the closed-chest model where reperfusion not can be verified visually. However, to account for minor hypoperfusion after reperfusion, the signal from [<sup>1-13</sup>C]alanine, [<sup>1-13</sup>C]lactate, and [<sup>13</sup>C]bicarbonate was normalized to the [<sup>1-13</sup>C]pyruvate signal. A reduced blood flow would decrease the supply of oxygen and inhibit cellular oxygen-dependent metabolism (bicarbonate reduction), whereas this metabolic shift is believed to increase the lactate conversion (Pasteur effect), and thus, the maintained balance between the anaerobic and the aerobic pathways (lactate-to-bicarbonate) suggests that the origin of the metabolic alterations is likely stemming from a reduced nutrient uptake in the apex region than from an oxygen-dependent altered metabolic conversion.

### Bicarbonate

The overall low [<sup>13</sup>C]bicarbonate signal postischemia could be a consequence of a general, low cardiac pyruvate dehydrogenase activity in the rat heart because of a metabolism shift toward the use of free fatty acids for energy production (17) and/or reduced anaerobic glucose metabolism. It is known that glucose and free fatty acids compete as substrates for energy production in the heart, and especially during fasting when fatty acid utilization increases and glucose metabolism and pyruvate dehydrogenase activity decreases (18, 19). The postischemic condition could mimic a “fasted” situation because the rat has been anesthetized for a long time before scanning. Alternatively, the overall low [<sup>13</sup>C]bicarbonate signal may be caused by a reduced carbonic anhydrase activity owing to, for example, ischemic stress that would increase the clearance of the rapidly diffusing (13) CO<sub>2</sub> by the blood and result in a reduced [<sup>13</sup>C]bicarbonate signal assuming that the end metabolic product is CO<sub>2</sub> and not H<sub>2</sub>CO<sub>3</sub><sup>-</sup> (20). Acidosis and increased flow would only, to a minor degree, reduce the [<sup>13</sup>C]bicarbonate signal. The glycolytic metabolism might fluctuate because of fluctuations in the glucose metabolism and thereby result in an unstable [<sup>13</sup>C]bicarbonate signal. A way to avoid fluctuations and keep a constant high plasma glucose levels could be to supply the animal with an oral glucose load or a direct infusion of glucose during the MR acquisition, which previously has shown to ensure a high myocardial glucose metabolism and a resulting high hyperpolarized [<sup>13</sup>C]bicarbonate signal (10, 11, 21). Looking back, imaging of the sham-operated rats could have been useful to further evaluate the effects of anesthesia on the metabolic signals.

### Coils

Two different coils were used, and data from both coils were included in the present study. A sensitivity profile of both coils is shown in Figure 1. The reason for using 2 coils was to evaluate their sensitivity and ensure coverage of the heart. Coverage of

the entire heart in the anterior–posterior direction (from coil surface into the animal) was not achieved by any of the coils. A strong signal was detected from the anterior wall closest to the coils, but low <sup>13</sup>C signal was detected from the posterior myocardial wall using both coils. Thus, signal differences were only assessed in the anterior wall, from a long-axis section. The length of the array coil elements is 42.5 mm in the head to tail direction, which is sufficiently long to cover the anterior wall. Because central positioning of the heart in the coil always was ensured, and because the heart was scanned both before and after ischemia with the same coil, the effects from coil sensitivity profiles on the evaluation from the anterior wall are considered to be negligible.

### Concluding Remarks

This study shows that regional metabolic changes following severe myocardial ischemia can be imaged in the *in vivo* rat heart by means of hyperpolarized [<sup>13</sup>C]pyruvate. Signal from both [<sup>13</sup>C]pyruvate, [<sup>13</sup>C]lactate, [<sup>13</sup>C]alanine, and [<sup>13</sup>C]bicarbonate could be detected before ischemia, and localized metabolic images of the anterior part of the rat myocardium using a surface coil, a 4-channel array coil, and a simple chemical shift imaging sequence could be produced. The decrease in the [<sup>13</sup>C]alanine and [<sup>13</sup>C]lactate signals in the ischemic region after 30 minutes of occlusion of the LAD coronary artery suggests that

the ischemic insult is severe, affecting not only the sensitive mitochondrial metabolism but also the cytosolic metabolism. It most likely reflects an overall depression of the cellular metabolism due to nutrient starvation, which is in agreement with the results of previous cardiac studies in pigs by use of hyperpolarized [<sup>13</sup>C]pyruvate (3, 6, 10). The metabolic finding was supported by the concomitant increase in the blood levels of troponin I and enhanced Gd signal using late enhancement MRI.

This study and previous studies using hyperpolarized <sup>13</sup>C MRI suggest that the technique has the potential to advance basic knowledge and improve diagnosis and prognosis of cardiac diseases. A successful translation of hyperpolarized <sup>13</sup>C MRI into the clinic will require an extensive understanding of the biological systems examined, which can be exploited in animal models such as the one presented here. The first hyperpolarized [<sup>13</sup>C]pyruvate MRI of a human heart was recently performed (22), proving the feasibility of the technique to acquire high signal-to-noise ratio metabolic images in human hearts. However, further technical advancements, such as increased polarization, coils with better sensitivity, and improved MR sequences to detect low <sup>13</sup>C signals, are still needed. In this study, [<sup>13</sup>C]pyruvate was used. In addition, other hyperpolarized substrates may offer alternative ways to study cardiac metabolism or specific chemical pathways affected by disease.

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Authors' Contributions: MHL carried out the animal experiments, including the heart surgery (LAD occlusion), and performed the MR scanning, collected the data, and drafted the manuscript; PM programmed and optimized the MR pulse sequences for the hyperpolarized studies and optimized the late enhancement imaging, constructed hyperpolarized image analysis tools, participated in the image analysis, and helped to draft the manuscript; CL constructed the hyperpolarized image analyzing tool, participated in data analysis, and helped to draft the manuscript; SAB helped with the hyperpolarized MRI scanning; JHAL helped coordinate the study, optimize the hyperpolarized MRI scanning and collect the data, interpret the results, and draft the manuscript; LVS coordinated the study and helped design the hyperpolarized <sup>13</sup>C MRI experiments, helped collect the data and draft the manuscript; OBP helped interpret the results and draft the manuscript; PÅ conceived and designed the study, helped coordinate the study, and helped to draft the manuscript.

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Conflict of Interest: The authors have no conflict of interest to declare.

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