Metabolic imaging of acute and chronic infarction in the perfused rat heart using hyperpolarised [1-13C]pyruvate

Daniel R. Ball\textsuperscript{a}, Rachel Cruickshank\textsuperscript{a}, Carolyn A. Carr\textsuperscript{a}, Daniel J. Stuckey\textsuperscript{b}, Philip Lee\textsuperscript{c}, Kieran Clarke\textsuperscript{a} and Damian J. Tyler\textsuperscript{a*}

Hyperpolarised \textsuperscript{13}C MRI can be used to generate metabolic images of the heart in vivo. However, there have been no similar studies performed in the isolated perfused heart. Therefore, the aim of this study was to develop a method for the creation of \textsuperscript{13}C metabolite maps of the perfused rat heart and to demonstrate the technique in a study of acute and chronic myocardial infarction. Male Wistar rat hearts were isolated, perfused and imaged before and after occlusion of the left anterior descending (LAD) coronary artery, creating an acute infarct group. In addition, a chronic infarct group was generated from hearts which had their LAD coronary artery occluded in vivo. Four weeks later, hearts were excised, perfused and imaged to generate metabolic maps of infused pyruvate and its metabolites lactate and bicarbonate. Myocardial perfusion and energetics were assessed by first-pass perfusion imaging and \textsuperscript{31}P MRS, respectively. In both acute and chronically infarcted hearts, perfusion was reduced to the infarct region, as revealed by reduced gadolinium influx and lower signal intensity in the hyperpolarised pyruvate images. In the acute infarct region, there were significant alterations in the lactate (increased) and bicarbonate (decreased) signal ratios. In the chronically infarcted region, there was a significant reduction in both bicarbonate and lactate signals. \textsuperscript{31}P-derived energetics revealed a significant decrease between control and chronic infarcted hearts. Significant decreases in contractile function between control and both acute and chronic infarcted hearts were also seen. In conclusion, we have demonstrated that hyperpolarised pyruvate can detect reduced perfusion in the rat heart following both acute and chronic infarction. Changes in lactate and bicarbonate ratios indicate increased anaerobic metabolism in the acute infarct, which is not observed in the chronic infarct. Thus, this study has successfully demonstrated a novel imaging approach to assess altered metabolism in the isolated perfused rat heart.

Keywords: perfused rat heart; metabolic imaging; hyperpolarised pyruvate; myocardial infarction

INTRODUCTION

With the recent enhancements in the MR sensitivity of the \textsuperscript{13}C nucleus achieved using dynamic nuclear polarisation (DNP) (1), it is now possible to complement structural data using MRI with global metabolic information in the myocardium (2–17). Work with several in vivo animal models has combined DNP with different imaging techniques (18–22), including chemical shift imaging (CSI) (23), to obtain metabolic maps of the heart using hyperpolarised [1-\textsuperscript{13}C]pyruvate (2,20,24–27). However, these studies have focused on in vivo models, with no work to date reported on the isolated perfused heart.

The isolated perfused rat heart (28) is ideal for the assessment of both physiological and pathological metabolism (29–39). It can be studied independent of hormonal and neuronal influences present in vivo, making causal changes in disease easier to determine. In addition, the isolated perfused rat heart model is devoid of surrounding tissues; metabolite signal contaminations from adjacent tissues, such as the liver and chest wall, are therefore removed. Rapid metabolic changes can be assessed after cardiac ischaemic interventions more quickly than is possible in vivo models. Higher signal-to-noise ratios (SNRs) and improved signal homogeneity can be obtained as a result of the closer placement of the radiofrequency (RF) coil to the heart. Better spectral separation and narrower linewidths are also possible as a result of the higher field strengths and narrower bore magnets generally used with the isolated perfused rat heart.

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Abbreviations used: CSI, chemical shift imaging; DNP, dynamic nuclear polarisation; \textit{H}\textsubscript{2}E, haematoxylin and eosin; LAD, left anterior descending; LDH, lactate dehydrogenase; Na\textsubscript{EDTA}, sodium ethylenediaminetetraacetate; PCr, phosphocreatine; PDH, pyruvate dehydrogenase; RF, radiofrequency; ROI, region of interest; RPP, rate pressure product; SNR, signal-to-noise ratio.

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The aim of the work described here was to develop a combined DNP and CSI protocol for application in the isolated perfused rat heart to generate metabolic images of hyperpolarised [1-13C]pyruvate and its downstream metabolites. Following infusion, hyperpolarised [1-13C]pyruvate is converted to [1-13C]lactate, via lactate dehydrogenase (LDH), [1-13C]alanine, via alanine aminotransferase, and [13C]bicarbonate, via pyruvate dehydrogenase (PDH) (5,13). It should be possible to generate maps of these metabolites in the isolated perfused rat heart and to use the metabolic images of [1-13C]pyruvate as a marker of tissue perfusion, [1-13C]lactate as a marker of anaerobic metabolism and [13C]bicarbonate as a marker of aerobic metabolism.

To demonstrate the utility of the developed DNP-CSI protocol, two different models of myocardial infarction were studied. An acute model, in which imaging was performed immediately before and following ex vivo occlusion of the left anterior descending (LAD) coronary artery, and a chronic model, in which LAD coronary artery occlusion was performed in vivo and the heart was perfused and imaged 4 weeks later (40–43). In all infarcted hearts, DNP-CSI data were used to generate cross-sectional maps of pyruvate, lactate and bicarbonate, which were compared with those from control perfused hearts. In addition, MR images acquired during the first pass of a gadolinium contrast agent were used to assess myocardial perfusion (44), using 13C nuclei were acquired with a single-channel, home-built, 20-mm birdcage RF coil. Those using 1Ho or 31P nuclei were acquired using a 20-mm, dual-tuned birdcage RF coil (Rapid Biomedical GmbH, Rimpar, Germany). Spectra and images were processed using jMRUI 2.2, Matlab 2011b (Mathworks, Natick, MA, USA) and ImageJ (W. Rasband, National Institutes of Health, Bethesda, MD, USA), as detailed below.

Materials and methods

[1-13C]Pyruvic acid and all unlabelled compounds were obtained from Sigma-Aldrich (Gillingham, Dorset, UK) and the trityl radical OX063 was obtained from Oxford Instruments Molecular Biotools (Abingdon, Oxfordshire, UK). All investigations conformed to Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act, 1986 (HMSO) and to institutional guidelines.

Software and equipment

For all studies, a vertical-bore, 11.7-T, wide-bore, MRI system (Magnex, Yarnton, Kidlington, Oxfordshire, UK) was used in combination with a Bruker Avance console (Bruker Medical, Ettlingen, Germany) running Paravision 2.1.1 and XWinNMR 2.6. Protocols using 13C nuclei were acquired with a single-channel, home-built, 20-mm birdcage RF coil. Those using 1H or 31P nuclei were acquired using a 20-mm, dual-tuned birdcage RF coil (Rapid Biomedical GmbH, Rimpar, Germany). Spectra and images were processed using jMRUI 2.2, Matlab 2011b (Mathworks, Natick, MA, USA) and ImageJ (W. Rasband, National Institutes of Health, Bethesda, MD, USA), as detailed below.

Perfusion protocol

Male Wistar rats were anaesthetised with a 1.3 mL/kg intraperitoneal injection of pentobarbital sodium (200 mg/mL, Euthatal) and, after the loss of pedal reflexes, a thoracotomy was performed and the heart was excised and placed into ice-cold Krebs-Henseleit buffer. The aorta was then cannulated and the heart was perfused in the Langendorff mode, initially under 65 mmHg perfusion pressure, and maintained at 37 °C. A drain was inserted into the left ventricle together with a polyethylene balloon to measure the heart rate and contractile function. The rate pressure product (RPP) was used as an index of cardiac function derived from the multiplication of the heart rate and the developed pressure.

A 20-mm NMR tube was placed over the heart and inserted into the bore of the 11.7-T magnet, whereby the heart was then subjected to a perfusion pressure of 85 mmHg. After a 5-min stabilisation period, a constant flow pump was set to deliver Krebs-Henseleit buffer at a flow rate of 15 mL/min. The buffer contained 118 mm NaCl, 4.7 mm KCl, 1.2 mm MgSO4·7H2O, 1.75 mm CaCl2·2H2O, 0.5 mm sodium ethylenediaminetetraacetate (Na2EDTA), 11 mm glucose, 25 mm NaHCO3 and 2.5 mm sodium pyruvate.

Sample preparation and dissolution

Aliquots (30 mg) of [1-13C]pyruvic acid were mixed with the trityl radical (OX063, 15 mm) and a trace amount of Dotarem (Guerbet, Villepinte, France), and placed in a HyperSense hyperpolariser (8) (Oxford Instruments Molecular Biotools). The samples were polarised at 94,128 GHz for 1 h and, following polarisation, the frozen [1-13C]pyruvic acid was dissolved with 6 mL of hot NaOH–EDTA–Tris buffer to neutralise the pH. Following dissolution, the hyperpolarised sodium pyruvate solution was added to 178 mL of Krebs-Henseleit buffer (without pyruvate) to yield a 2.5 mM hyperpolarised sodium [1-13C]pyruvate solution (previously described in ref. (8)). This solution was then delivered to the heart as a bolus over 120 s, and the CSI protocol (detailed below) was acquired.

Parameter optimisation

Prior to the commencement of the myocardial infarction studies, a B1 field map was constructed using the 13C birdcage RF coil to ensure that the sensitive region of the coil was homogeneous (20). To ascertain the ideal flow rate to the heart, the maximum hyperpolarised [1-13C]pyruvate signal amplitude was investigated using a simple pulse-acquire acquisition at flow rates of 5, 10, 15 and 20 mL/min.

Following this, several parameters relevant to the CSI acquisition protocol were optimised. The effect of variation in the acquisition flip angle (n = 3; 22.5°, 45°, 67.5° and 90°) and TR (n = 3; 200, 400, 600, 800, 1000 and 1200 ms) on the maximum signal amplitude of [1-13C]pyruvate, [1-13C]lactate and [13C]bicarbonate was then investigated. These acquisitions were performed using the CSI protocol, but with the gradient amplitudes set to zero, thus allowing the temporal variation in signal amplitude to be explored.

Infarct studies

Two groups of isolated hearts were investigated: one in which hearts were isolated and a myocardial infarction was induced during perfusion (n = 9) to investigate acute metabolic changes, and a second that was surgically infarcted in vivo (n = 4) and then isolated and perfused 4 weeks after infarction to investigate chronic metabolic changes. For the acute study group, hearts from healthy male Wistar rats (body weight~300 g) were assessed as described below. These same hearts were then infarcted by occlusion of the LAD coronary artery and the assessment was immediately repeated (<10 min between occlusion and metabolic imaging). All hearts recovered contractile function.
Data acquisition protocol

An initial localiser scan was performed to ensure that the heart was placed within the most sensitive region of the RF coil and shimming was performed to give a proton linewidth of approximately 50 Hz. A high-resolution axial scan (TR = 100 ms; TE = 1.280 ms; slice thickness, 1 mm; field of view, 24 × 24 mm²; matrix, 128 × 128; averages, 8) allowed orientation of the heart with respect to subsequently acquired data. Following polarisation and dissolution, the hyperpolarised [1-13C]pyruvate solution was infused into the heart and a CSI acquisition was performed. The CSI protocol consisted of an in-house-developed elliptical CSI acquisition with the following parameters: TR = 0.5 s; flip angle, 45°; slice thickness, 5 mm; field of view, 24 × 24 mm²; matrix, 8 × 8; averages, 2. This provided a metabolic distribution map of [1-13C]pyruvate and its metabolites lactate and bicarbonate.

Subsequently, a first-pass perfusion scan was performed to quantify the degree of myocardial perfusion and, where relevant, to confirm the presence and location of the infarcted region. The first-pass protocol, derived from previous work (44), comprised a fast gradient echo sequence to acquire an image every 128 ms with an in-plane spatial resolution of 78 μm (TR = 2 ms; slice thickness, 1 mm; field of view, 20 × 20 mm²; 128 images). Six seconds after the start of this sequence, 25 μL of gadodiamide (0.5 mm Omniscan, GE Healthcare, Chalfont St Giles, Buckinghamshire, UK) was administered via a prefilled drug line. The acquisition of 128 images gave a total imaging time of ~16 s. Following this scan, the global cardiac energetic status was measured using a fully relaxed 31P spectrum (TR = 15 s; flip angle, 90°; 40 averages; bandwidth, 49.39 ppm; number of points, 2048) (45).

On completion of all acquisitions, chronically infarcted hearts were frozen in a water-based glycol mixture for later histological analysis to determine the extent of cell necrosis. Slices were stained with haematoxylin and eosin (H&E) as well as picrosirius red (46,47).

Data analysis

The high-resolution axial slice was used for heart orientation when analysing gadolinium and CSI data. For both sets of data, four regions of interest (ROIs) were created: one over the infarct region, two in the peri-infarct zones and one in the remote inferior region of the left ventricle. The ROIs were fixed in size to be 2 mm × 2 mm and were identical for the first-pass perfusion and metabolic images. The infarct ROI was placed in the centre of the region of hypointensity on the first-pass perfusion images; the peri-infarct ROIs were placed immediately to either side of this area of hypointensity; the remote ROI was placed directly opposite this area of hypointensity. The two peri-infarct ROIs were averaged for analysis. An outline of the heart from the epicardial border of the high-resolution scan was overlaid onto the gadolinium and CSI images to further aid orientation.

The data acquired using the CSI protocol were zero filled to match the resolution of the proton images and Fourier transformed using software written in-house in Matlab R2011b (Mathworks). Following Fourier transformation, the frequencies of the pyruvate, lactate and bicarbonate resonances were selected, and the area under each resonance was calculated on a pixel-by-pixel basis to allow the generation of metabolic images. The mean signal intensity within the four selected ROIs was then calculated for each metabolic image.

The gadolinium contrast agent scans were initially processed in ImageJ (W. Rasband, National Institutes of Health), where the mean signal intensity in the four ROIs was recorded for each of the 128 frames of the acquisition. The time-course data produced were subsequently fitted to a sigmoidal function to allow the calculation of the signal increase observed on addition of gadolinium. 31P spectra were processed and quantified using jMRUI (45).

To assess the variability in the metabolite signal levels within and between individual hearts, coefficients of variation were calculated for the four ROIs in the data acquired from the acutely infarcted hearts before occlusion of the LAD coronary artery.

Statistical analysis

The data are reported as the mean ± SEM and all statistical analysis was performed using GraphPad Prism (GraphPad, La Jolla, CA, USA). Statistical significance between the pre-occlusion and post-occlusion data for each selected ROI in the acute infarct model was assessed using a paired Student’s t-test. Statistical significance between the different ROIs in the chronic infarct model was performed using a one-way analysis of variance and a post-hoc Bonferroni multiple comparison test. Statistical significance was considered at the p < 0.05 level.

RESULTS

CSI protocol optimisation

Variation of the buffer flow rate (Fig. 1A) showed that a higher flow rate led to earlier arrival of the hyperpolarised [1-13C]pyruvate at the heart, resulting in a higher pyruvate signal amplitude because of the shorter hyperpolarisation decay time. Based on these results, and previous measurements of flow in the Langendorff perfused heart, a flow rate of 15 mL/min was chosen as a suitable compromise between enhanced signal and an appropriate workload for the isolated perfused heart.

Variation of the applied flip angle (Fig. 1B) demonstrated no significant relationship between pyruvate, bicarbonate or lactate signal amplitudes and the applied flip angle. There appeared to be a trend towards a higher hyperpolarised pyruvate signal amplitude and lower metabolite signal amplitude with increasing flip angle. This may be expected, as higher flip angles would lead
Figure 1. (A) Variation in the signal amplitude of hyperpolarised [1,13C]pyruvate with respect to flow rate from the cannula into the perfused heart. There is a direct correlation between the flow rate and maximum signal amplitude. (B) Variation in the signal amplitude with respect to the flip angle for both pyruvate and its metabolites. No significant correlation was observed and a flip angle of 45° was chosen, given the trend for increasing pyruvate and decreasing metabolite signals. (C) Correlation between the repetition time (TR) and signal amplitude for each of the metabolite signals. As expected, a positive correlation was obtained in each case.
to increased signal intensity from the in-flowing pyruvate, but would destroy the enhanced polarisation, therefore limiting the signal from downstream metabolites. As a result, 45° was chosen as the flip angle for this study, providing the best compromise between pyruvate and metabolite signal intensities.

A range of TR values between 200 and 1200 ms was subsequently investigated (Fig. 1C), and a significant increase in the maximum signal intensities of both pyruvate and downstream metabolite was observed. Again, this would be expected, as an increased TR would allow more time for the inflow of new hyperpolarised pyruvate and subsequent conversion into lactate and bicarbonate. However, increased TRs also lead to an increase in the total CSI time and therefore increased decay of the hyperpolarised signal in the later stages of the image acquisition. As a result, a repetition time of 500 ms was chosen to maintain imaging times at approximately 1 min, whilst allowing the acquisition of two signal averages and an 8 x 8 acquisition matrix, thereby maintaining relatively high signal amplitudes from the hyperpolarised pyruvate and its metabolites.

**Acute infarct model**

Figure 2 shows example images acquired before (Fig. 2A–E) and after (Fig. 2F–J) occlusion of the LAD coronary artery in the Langendorff perfused rat heart. Figures 2A and 2F represent the high-resolution axial images and demonstrate the ROIs selected as infarct (I), peri-infarct (P) and remote (R) tissue for subsequent analysis. Figures 2B and 2G show single timeframe acquisitions from the end of the first-pass perfusion scans. There is a clear area of low signal intensity in the region of the heart supplied by the occluded LAD artery. Figures 2C–E and 2H–J show metabolite maps of pyruvate, bicarbonate and lactate from before and after LAD artery occlusion, respectively. In the images generated before LAD artery occlusion, there is a reasonable level of signal homogeneity around the circumference of the left ventricle, although there is a slight elevation in signal intensity in the septal wall, potentially caused by contamination from the right ventricular cavity/myocardium. The analysis of this signal homogeneity is presented in Table 1, with coefficients of variation for the signal intensity presented to highlight the level of signal variation both within and between individual hearts.

![Figure 2](image.png)

**Table 1. Coefficients of variation for the signal intensity measured in the four regions of interest (ROIs) in the acutely infarcted hearts before occlusion of the left anterior descending (LAD) coronary artery. Coefficients of variation are presented to highlight the level of signal variation both within and between individual hearts.**

|                    | Pyruvate (%) | Lactate (%) | Bicarbonate (%) |
|--------------------|--------------|-------------|-----------------|
| Within hearts      | 21.3         | 17.5        | 16.7            |
| Between hearts     | 26.1         | 33.5        | 27.3            |

In the post-occlusion images, there is a clear area of reduced signal in the pyruvate and bicarbonate images, which corresponds to the infarct area, as demonstrated by the low signal intensity in the gadolinium contrast image. Conversely, the post-occlusion lactate image shows an elevated signal intensity, which correlates with the infarct area.

Quantitative analysis of the gadolinium and CSI images for all hearts is shown in Figure 3. A significant reduction in pyruvate signal intensity in the infarct region was observed (Fig. 3A), which corresponded to a significant reduction in gadolinium-induced signal increase (Fig. 3D), indicating a decrease in buffer perfusion to the infarct region. No difference in perfusion to the peri-infarct or remote regions was observed in either the pyruvate images or the gadolinium contrast agent scans. A significant reduction in bicarbonate signal intensity (Fig. 3B) was observed in the infarct region, but not in the peri-infarct or remote regions. To account for the reduced perfusion of the hyperpolarised pyruvate to the infarct region, the bicarbonate signal intensity was normalised to that of pyruvate. No significant change in the normalised bicarbonate signal was seen in any region, before or after occlusion of the LAD artery (Fig. 3E).

No significant differences in the lactate signal intensity were observed for any ROI, before or after occlusion. However, following normalisation to the pyruvate signal intensity to account for the reduced perfusion to the infarct region, a significant increase in lactate signal intensity was observed in the infarct region (Fig. 3F). To provide an index of the relative...
balance between anaerobic and aerobic metabolism within the different regions, Figure 4 shows the ratio of the lactate to bicarbonate signal intensities for the different ROIs. A highly significant increase in the ratio was seen in the infarct region, indicating an increased reliance on anaerobic metabolism in the area of tissue no longer perfused via the LAD artery.

Following occlusion of the LAD artery, there was a significantly reduced RPP [Table 2, \((27 \pm 6) \times 10^3 \text{mmHg/min}, p < 0.05\)] and PCr/ATP ratio (Table 2, \(2.0 \pm 0.1 \text{versus} 2.3 \pm 0.1, p = 0.2\)), indicating reduced cardiac function, but maintenance of normal global cardiac energetics, in the acutely infarcted perfused rat heart.

### Chronic infarct model

The CINE protocol used for in vivo chronic infarct verification showed a significantly reduced ejection fraction in the infarcted hearts relative to control scans taken 1 week prior to surgery \((n = 4, 56 \pm 4\% \text{versus} 78 \pm 3\%, p < 0.05)\). When perfused, the hearts showed a significantly reduced RPP [Table 2, \((27 \pm 6) \times 10^3 \text{mmHg/min}, p < 0.05\)] and PCr/ATP ratio (Table 2, \(2.0 \pm 0.1 \text{versus} 1.6 \pm 0.1, p < 0.05\)] when compared with the acutely infarcted hearts pre-occlusion. Taken together, these data demonstrate a significant functional and energetic impairment in the chronically infarcted hearts.

Figure 5 shows example images (Fig. 5A, B, D–F) and quantitative analysis (Fig. 5C, G–I) of the chronically infarcted Langendorff perfused rat heart. Figure 5A shows the high-resolution axial scan and indicates the selected ROIs for this example heart. The first-pass perfusion scan in Figure 5B highlights the reduction in gadolinium-induced signal increase seen in the infarcted region, which is quantified in Figure 5C, confirming a reduction in perfusion to the infarcted area relative to the remote and peri-infarct regions. Figure 5D–F shows example pyruvate, bicarbonate and lactate images from this heart. There is an obvious reduction in signal amplitude for both pyruvate and its downstream metabolites in the infarct region. This is confirmed by the quantitative analysis shown in Figure 5G–I, which indicates reductions in pyruvate, bicarbonate and lactate signal intensities in the infarct region relative to the remote and peri-infarct regions. This implies a significant reduction in perfusion and, as a consequence, reduced metabolism in the infarcted region of the heart. The anaerobic/aerobic ratio, as measured for the acute heart, revealed no significant differences in the chronic model (Fig. 6).

H&E histology staining revealed necrosis in the chronically infarcted hearts (Fig. 7) via the visualisation of delocalised nuclei and a reduced tissue mass in the infarcted area relative to...
controls. In addition, a large number of collagen deposits in the infarct region could be seen, as visualised with picrosirius red staining.

**DISCUSSION**

In this study, an optimised DNP-CSI protocol has been developed for the isolated perfused rat heart. The ability to visualise the metabolic distribution and to map it to the anatomical layout of the heart has been demonstrated using two different infarct models. Clear differences in the metabolic response within the acutely and chronically infarcted heart were observed and were correlated with alterations in perfusion, function and energetics. Images with a high spatial resolution and smaller slice thickness than have been demonstrated *in vivo* were produced in this study. This was particularly relevant for a rodent infarct model, where it may be difficult to distinguish healthy myocardium from ischaemic tissue in a thick slice, given the small size of the rat heart. Further, this study allowed the same heart to be investigated before and after an intervention, enabling the acquisition of control and ‘disease’ data within the same subject. Changes in metabolism induced minutes after an ischaemic intervention were assessed; examination of the metabolic changes of the heart minutes after infarct generation would be exceptionally difficult in an *in vivo* model. The DNP-CSI protocol generated can be easily incorporated into standard perfused heart protocols, and the examples presented here demonstrate the ease with which the DNP-CSI acquisition can be combined with other

Table 2. Cardiac energetic and functional status. There is a significant decrease in the phosphocreatine (PCr)/ATP ratio when comparing controls with chronic infarcts and a significant decrease in the rate pressure product (RPP) when comparing controls with both infarct groups (*p < 0.05)

|                          | PCr/ATP (a.u.) | Heart rate (beats/min) | RPP (×10³ mmHg/min) |
|--------------------------|---------------|------------------------|---------------------|
| Control (pre-occlusion)  | 2.0 ± 0.1     | 290 ± 30               | 27 ± 6              |
| Acute infarct (post-occlusion) | 2.3 ± 0.1     | 310 ± 40               | 18 ± 3*             |
| Chronic infarct (*in vivo occlusion*) | 1.6 ± 0.1*    | 300 ± 30               | 16 ± 7*             |

Figure 5. Example images and quantitative analysis in the chronically infarcted hearts. (A) High-resolution axial scan. (B) Final frame from the first-pass gadolinium perfusion scan. (C) Significant reduction in perfusion as assessed by gadolinium contrast. (D–F) Example pyruvate, bicarbonate and lactate images, respectively. A significant reduction is seen in absolute pyruvate (G), bicarbonate (H) and lactate (I) signal intensities in the infarct area when compared with the remote region. *p < 0.05, **p < 0.01.
acquisitions (such as late gadolinium perfusion and $^{31}$P spectroscopy) to generate a comprehensive assessment of the perfused heart.

The work developed here made use of a spectroscopic elliptical CSI sequence and, before acquiring data in the infarcted heart, it was first necessary to optimise the DNP-CSI protocol for small-volume imaging in the perfused heart system. Although previous studies (24,49) have performed DNP-CSI in vivo, the acquisition of data from the perfused heart is different in several ways.

(1) In the in vivo situation, there is generally a single bolus injection, and therefore a single source of hyperpolarised $[1-^{13}$C$]$pyruvate, whose decay can be predicted in a straightforward manner with respect to both $T_1$ and RF pulse considerations. In comparison, the perfused heart has a constant influx of hyperpolarised pyruvate throughout the acquisition. This fresh influx of hyperpolarised pyruvate allows for much larger flip angles to be used for RF excitation, and results in subsequently higher metabolite signals. In the in vivo experiments, more complex RF pulse sequences are required to avoid the destruction of the hyperpolarised pyruvate signal too quickly(20).

(2) Frequently, when imaging in vivo, surface RF coils have been utilised. This generally results in a decrease in sensitivity with distance from the coil and requires correction for the inhomogeneous flip angle experienced (25). In the isolated perfused heart, the heart can be located inside a birdcage RF coil, thereby ensuring that the entire heart is within the homogeneous region of the coil. Even with the use of volume coils in vivo, the chest wall can contaminate the signal, and the close proximity of air spaces within the lungs can increase the spectral linewidth.

Following optimisation, the DNP-CSI protocol allowed the generation of high-SNR metabolic images, which clearly delineate the structure of the left ventricle (Fig. 2). However, this was only possible for metabolites in which the $^{13}$C label was significantly incorporated. In our experiments, $[1-^{13}$C$]$alanine did not fit this criterion, generating low-SNR images, which were therefore excluded from our subsequent analysis. In the light of in vivo studies, which have either demonstrated the presence of alanine (16,22) or have chosen not to process it (2,26) in the cardiac region, the low SNR warrants comment. Studies carried out in pigs tend to show low levels of alanine in comparison with lactate and bicarbonate, yet recent work in the in vivo rat heart (22) appears to show alanine levels comparable with those of bicarbonate. It is interesting that, relative to the myocardium, a large contribution of alanine appears to originate from the chest wall. This chest wall contribution cannot be the case in the isolated perfused heart, providing one possible reason for the low alanine signal seen in this study. In addition, the thick slices needed with current and future rodent in vivo studies are likely to acquire some contamination from the liver, despite respiratory motion compensation. Even with the acquisition of high-SNR images provided by pyruvate, bicarbonate and lactate in the perfused heart, there was still a residual level of inhomogeneity in the images generated in this study. This may be a result of inherent regional differences in perfusion within the different territories of the myocardium. Quantitative assessment showed that the level of inhomogeneity within individual hearts tends to be slightly lower than that seen between hearts (Table 1).

To demonstrate the utility of the developed protocol in the study of the diseased heart, two different models of myocardial infarction were investigated. In both models, LAD artery occlusion was used to reduce perfusion to the anterolateral region of the heart, thereby creating an infarct. The reduction in flow to the LAD artery territory was clearly visible using a first-pass

Figure 6. The ratio of lactate to bicarbonate in the chronic infarct model. The ratio represents an index of the balance between anaerobic and aerobic metabolism, and shows no significant differences across any region of the myocardium.

Figure 7. Example histological sections from the chronically infarcted heart. (A) Haematoxylin and eosin (H&E) staining revealing, in blue, the nuclei that have been delocalised from the membrane. (B) Collagen stained in red using picrosirius red. The images are 10x magnifications, 15 mm in width. Both images clearly show the formation of an infarct in the top left quadrant of the heart.
gadolinium perfusion acquisition, and resulted in a significant reduction in the gadolinium-induced signal increase in both infarct models. This reduced perfusion was also apparent in the corresponding [1-13C]pyruvate images, demonstrating the utility of hyperpolarised pyruvate as an index of myocardial perfusion.

In the acute infarct model, LAD artery occlusion led to a significant decrease in cardiac function (Table 2), but no alteration in the PCR/ATP ratio, implying that, despite the reduced tissue perfusion, the global cardiac energetic status was able to be maintained, at least for a limited time period. In the acutely ischaemic environment of this model, there should be a reduction in oxidative metabolism and a concomitant increase in anaerobic metabolism (50). A decrease in the bicarbonate signal, as a result of a decrease in PDH flux, and an increase in the lactate signal in the infarct region would therefore be expected. In support of this, a significant decrease in the absolute bicarbonate signal in the acutely infarcted region was observed. However, although there was a trend for the absolute lactate signal to increase, this was not significant (p = 0.2). The interpretation of these data is complicated by the change in perfusion to the infarcted area and the subsequently reduced supply of [1-13C]pyruvate. The simplest ways to account for this are either to eliminate the effect of perfusion by calculating the ratio of lactate to bicarbonate signal intensities or to normalise the metabolite data with respect to the hyperpolarised pyruvate.

The ratio of lactate to bicarbonate signal intensities provides an index of the balance between anaerobic and aerobic metabolism and, as expected, this ratio was increased significantly in the acutely infarcted heart. The normalised lactate signal was also seen to be increased significantly, demonstrating an increased anaerobic metabolism. However, the underlying mechanism may not be as simple as an increased flux through LDH. Experimental evidence has shown that, in the presence of a large amount of isotopically unlabelled lactate, as would be the case in infarcted glycolytic cardiac cells, an infusion of hyperpolarised [1-13C]pyruvate will result in a greater than expected hyperpolarised lactate signal (51). This implies that the lactate seen is essentially a reflection of the lactate already present in the myocardium.

The normalised bicarbonate data, however, do not reveal a significant decrease as might be expected, suggesting that the reduction in aerobic metabolism in the infarct region is proportional to the reduction in pyruvate delivery to the area. In the chronic infarct model, there was a significant reduction in both cardiac function and energetics, resulting from significant structural remodelling of the heart. This was seen in combination with a significant reduction in perfusion to the infarct region (as assessed by both gadolinium contrast and pyruvate signal intensity) and reductions in the signal intensity of both bicarbonate and lactate. The absolute decrease in metabolite levels can be explained by the decrease in pyruvate flow to the area and the decrease in the number of cells in the infarct area that are capable of pyruvate metabolism (Fig. 6). Normalising these data to the pyruvate signal showed no change, which indicates a level of remaining oxidative metabolism, which is reduced in proportion to the reduced pyruvate delivery to the area. This could be a result of the incorporation of some healthy tissue within the myocardium above and below the infarcted area because of the slice thickness of the DNP-CSI acquisition (5 mm). Although this slice thickness is still smaller than that possible in vivo, improved hardware should allow for a smaller slice thickness and therefore permit a more detailed metabolic evaluation of the chronic infarct model, in addition to that already provided using the DNP-CSI technique with the acute infarct model. Further, the implementation of either multi-slice or three-dimensional imaging techniques could help to clarify this issue (26,44).

For the first time, a DNP-CSI protocol in the perfused rat heart has been demonstrated, and allows the combination of metabolic imaging using DNP-CSI with all the advantages of the isolated perfused heart. Metabolism can be elucidated independent of hormonal and neuronal influences, revealing the true contribution of ischaemia to PDH flux. Metabolic contamination, both from the ventricular cavity and nonmyocardial tissue, problematic in vivo, is removed in the isolated perfused heart, to allow for the accurate metabolic assessment of the myocardium. DNP-CSI in the isolated heart can therefore be a useful tool for the visualisation of metabolic alterations in the diseased heart.

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