Hyperpolarized $^{13}$C magnetic resonance reveals early- and late-onset changes to in vivo pyruvate metabolism in the failing heart

Marie A. Schroeder$^{1,2,*}$, Angus Z. Lau$^{1,3}$, Albert P. Chen$^4$, Yiping Gu$^1$, Jeevan Nagendran$^5$, Jennifer Barry$^1$, Xudong Hu$^6$, Jason R.B. Dyck$^5$, Damian J. Tyler$^2$, Kieran Clarke$^2$, Kim A. Connelly$^{1,6}$, Graham A. Wright$^{1,3}$, and Charles H. Cunningham$^{1,3}$

$^1$Sunnybrook Health Sciences Centre, 2075 Bayview Avenue, Room M326A, Toronto, Ontario M4N 3M5, Canada; $^2$Department of Physiology, Anatomy & Genetics, University of Oxford, UK; $^3$Department of Medical Biophysics, University of Toronto, Canada; $^4$GE-Healthcare, Toronto, Canada; $^5$Departments of Pediatrics and Pharmacology, University of Alberta, Canada; and $^6$Keenan Research Centre in the Li Ka Shing Knowledge Institute, St. Michael’s Hospital and University of Toronto, Toronto, Canada

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## Aims

Impaired energy metabolism has been implicated in the pathogenesis of heart failure. Hyperpolarized $^{13}$C magnetic resonance (MR), in which $^{13}$C-labelled metabolites are followed using MR imaging (MRI) or spectroscopy (MRS), has enabled non-invasive assessment of pyruvate metabolism. We investigated the hypothesis that if we serially examined a model of heart failure using non-invasive hyperpolarized $[^{13}\text{C}]$pyruvate with MR, the profile of in vivo pyruvate oxidation would change throughout the course of the disease.

## Methods and results

Dilated cardiomyopathy (DCM) was induced in pigs ($n=5$) by rapid pacing. Pigs were examined using MR at weekly time points: cine-MRI assessed cardiac structure and function; hyperpolarized $[^{2}\text{C}]$pyruvate was administered intravenously, and $^{13}$C MRS monitored $[^{13}\text{C}]$glutamate production; $^{31}$P MRS assessed cardiac energetics [phospho-creatine (PCr)/ATP]; and hyperpolarized $[^{1}\text{C}]$pyruvate was administered for MRI of pyruvate dehydrogenase complex (PDC)-mediated pyruvate oxidation via $[^{13}\text{C}]$bicarbonate production. Early in pacing, the cardiac index decreased by 25%, PCr/ATP decreased by 26%, and $[^{13}\text{C}]$glutamate production decreased by 51%. After clinical features of DCM appeared, end-diastolic volume increased by 25%, PCr/ATP decreased by 26%, and $[^{13}\text{C}]$glutamate production decreased by 51%. Pyruvate dehydrogenase kinase 4 protein increased by two-fold, and phosphorylated Akt decreased by half. Peroxisome proliferator-activated receptor-$\alpha$ and carnitine palmitoyltransferase-1 gene expression decreased by a half and a third, respectively.

## Conclusion

Despite early changes associated with cardiac energetics and $^{13}$C incorporation into the Krebs cycle, pyruvate oxidation was maintained until DCM developed, when the heart’s capacity to oxidize both pyruvate and fats was reduced. Hyperpolarized $^{13}$C MR may be important to characterize metabolic changes that occur during heart failure progression.

## Keywords

Magnetic resonance
Introduction

There is increasing evidence that changes in metabolic substrate utilization and depleted myocardial energetic reserve may contribute to LV dysfunction in patients with heart failure. Abnormal myocardial energy metabolism has been identified in heart failure patients using \(^{31}\)P magnetic resonance spectroscopy (MRS) and positron emission tomography (PET). Substrate utilization in the failing heart has been associated with a ‘fetal’ pattern of metabolic gene expression that results in the preferential use of carbohydrates over free fatty acids (FFAs) for ATP production. This topic remains controversial, however, as other studies have reported that the failing heart preferentially takes up FFAs and that the capacity for carbohydrate uptake and/or oxidation in heart failure is reduced, associated with insulin resistance. It has also been shown that the relative utilization of fatty acids and glucose shifts depending on the aetiology and stage of disease.

The controversy over substrate metabolism in heart failure may originate from the limitations of standard methods for assessing myocardial metabolism. For example, in vitro measurements of total protein or mRNA content do not necessarily reflect metabolic activity, and enzyme assays performed in homogenized tissue samples may be misleading owing to the maximal/unphysiological substrate and hormone levels used in the assay, compared with in vivo. Radiolabelled tracer imaging techniques, including PET and single photon emission computed tomography (SPECT), cannot distinguish between the injected tracer and its downstream metabolic products and they use ionizing radiation. To understand the timing and consequences of switches in substrate metabolism during heart failure, and potentially to use those switches to diagnose disease severity and optimize treatment, a non-invasive method capable of serially monitoring cardiac metabolism is required.

Magnetic resonance imaging and spectroscopy (MRI and MRS) have long been used to monitor cardiac structure and function non-invasively at repeated times and various stages of disease. The application of MR for metabolic imaging, however, has been limited by intrinsically low sensitivity. Hyperpolarization using the dynamic nuclear polarization (DNP) technique is a process that can yield >10,000-fold signal increases in MR-active nuclei. When used with MRI and MRS, hyperpolarized \(^{13}\)C-labelled tracers allow non-invasive visualization of normal and abnormal metabolism.

We hypothesized that, if we serially examined a model of heart failure using non-invasive hyperpolarized \(^{13}\)C pyruvate with MR, the pattern of pyruvate oxidation by the enzyme complex pyruvate dehydrogenase (PDC) and the Krebs cycle would change throughout the course of the disease. Furthermore, by using hyperpolarized \(^{13}\)C MR alongside MR-based measurements of cardiac energetics, structure, and contractile function, and in vitro measurements of ATP content and gene/protein expression, we aimed to enhance our understanding of how altered metabolic fluxes contribute to heart failure pathogenesis.

To test our hypothesis, we examined a porcine pacing model of dilated cardiomyopathy (DCM) using clinically applicable hyperpolarized \(^{13}\)C MRS and MRI methods, with the tracers \([1,13\text{C}]\)pyruvate and \([2,13\text{C}]\)pyruvate. Serial MR was applied for 0.5 mmol/kg of hyperpolarized \(^{13}\)C pyruvate oxidation via PDC. This work has provided the first evidence that metabolic imaging using hyperpolarized \(^{13}\)C MR may be a useful tool to diagnose disease severity and optimize treatment for heart failure patients, an important result as clinical application of hyperpolarized \(^{13}\)C MR in cardiovascular patients is positioned to occur in the near future.

Methods

Study overview

Dilated cardiomyopathy was induced in female Yorkshire pigs (n = 5, 20 kg at baseline, 1 month old) by chronic rapid right ventricular (RV) pacing. After allowing pigs to recover from pacemaker implantation for >1 week, pacemakers were set to beat at 188 b.p.m. until pigs developed heart failure. At baseline and at weekly intervals throughout the duration of the pacing protocol, MR was used to examine in vivo cardiac physiology in each pig (details below). Pigs were sacrificed at the first MR examination point at which they already displayed clinical signs of heart failure, including discoloured skin and mucosal membranes, dyspnoea, pulmonary oedema, myocardial dilatation, ascites, and peripheral oedema.

All MR experiments were performed on a GE MR750 3T MR scanner. After each pig was positioned in the magnet, proton cine-MRI images were acquired. During cine-MRI, the \([2,13\text{C}]\)pyruvate was hyperpolarized, and venous blood from the pig was taken for biochemical analyses. Once cine images were acquired, a dose of hyperpolarized \([2,13\text{C}]\)pyruvate was dissolved and infused into the ear vein while \(^{13}\)C MR spectra were acquired. Next, in the interval during which \([1,13\text{C}]\)pyruvate was hyperpolarized, \(^{31}\)P spectra were acquired. Finally, hyperpolarized \([1,13\text{C}]\)pyruvate was infused while \(^{13}\)C metabolic images were acquired.

Dynamic nuclear polarization with dissolution was used to generate both aqueous hyperpolarized \([2,13\text{C}]\)pyruvate (for MRS experiments) and \([1,13\text{C}]\)pyruvate (for MRI experiments). For both sets of experiments, 0.05 mmol/kg of hyperpolarized \([13\text{C}]\)pyruvate was injected over 15 s into the right ear vein, and scanning was initiated at the beginning of the infusion.

An overview of the experimental protocol is presented in Figure 1. A description of each MR acquisition is detailed below, and the procedures used for \([1,13\text{C}]\)pyruvate polarization and dissolution, MR data analysis, and for biopsy and heart tissue analyses are described further in the Supplementary material. Primer sequences designed in-house and used for real-time quantitative PCR (qRT-PCR) are given in Supplementary material, Table S2. All animal experiments were performed between 13:00 and 17:00 h and were carried out under a protocol approved by the institutional animal care and use committee.

Magnetic resonance imaging and spectroscopy

Proton cine-magnetic resonance imaging

To assess cardiac structure and function, cardiac-gated breath-held steady-state free precession (SSFP) cine images were acquired in the...
Carbon-13 magnetic resonance spectroscopy

Upon infusion of [2-13C]pyruvate, MR spectra were acquired to follow Krebs cycle-mediated conversion into [5-13C]glutamate. A slice-selective, cardiac-gated pulse-and-acquire sequence [slice thickness 10 cm, nominal fractional anisotropy (FA) = 10°] was used to acquire data, using a custom-built transmit/receive 13 cm 13C surface coil. Spatial localization was provided by a combination of the small tip-angle slice-selective sinc excitation pulse and the placement of the 13C surface coil on the chest, over the region where the heart was located. One spectrum (2048 spectral points, 5 kHz bandwidth) was acquired cardiac gated to every three R–R intervals to acquire one time point approximately every 2 s.

Phosphorus-31 magnetic resonance spectroscopy

31P two-dimensional chemical shift imaging (2D-CSI) data were acquired from a 3 cm slice in short-axis view using a cardiac-gated pulse-acquire pulse sequence (16 x 16 matrix, 3 cm in-plane resolution, 45° tip angle, TR = 2R/R, ~1.5 s, four averages), and a 13 cm 31P transmit/receive surface coil.

Carbon-13 magnetic resonance imaging

A chemical shift-specific, cardiac and respiratory-gated 13C MRI sequence was used to image [1-13C]pyruvate, [13C]bicarbonate, and [1-13C]lactate with 9 mm in-plane spatial resolution in two 1 cm slices. Metabolite images were acquired with 2.5 s temporal resolution per set of slices to capture both the time course of the [1-13C]pyruvate bolus and its subsequent metabolism. A spectral–spatial pulse was used to select the appropriate resonance for imaging in each frame. Two short-axis images at mid-chamber and apical positions were acquired at end-expiration in diastole. A total of 35 imaging frames were acquired over a total scan time of 1.5 min, as follows: 10 frames (7.5° tip angle) to capture the first pass of the [1-13C]pyruvate bolus were acquired, followed by an interleaved set of 25 frames corresponding to bicarbonate (90° tip angle), lactate (90°), and pyruvate (45°).

Data analysis

Pigs took between 4 and 6 weeks of pacing to develop clinical signs of DCM. To allow comparison of all parameters measured for each pig, data were grouped into three time periods. Grouping depended solely on the pacing duration and was performed blinded to data analysis. The first period (early) reflected the physiological changes due to early pacing, and reported an average of data acquired after 1–2 weeks.
of pacing. The second period (moderate) reflected a stage of subclinical cardiac dysfunction, and reported an average of data acquired after 2–5 weeks of pacing. The third period (DCM) reflected overt heart failure. Only data taken from the terminal examination point, after our endpoint had been reached, were included in this group (4–6 weeks of pacing).

Proton cine-magnetic resonance imaging

The LV epicardial borders, and the LV and RV endocardial borders were manually outlined in end-diastolic and end-systolic frames. Global end-diastolic volume (EDV), end-systolic volume (ESV), wall thickness, EF, and cardiac output (CO) were calculated. All parameters were indexed to pig body surface area.

Carbon-13 magnetic resonance spectroscopy

For each dynamic series of MR spectra, relative [5-13C]glutamate production was measured by calculating the ratio of the maximum [13C]bicarbonate and [1-13C]pyruvate signal per unit volume, normalized to the mean [1-13C]pyruvate signal per unit volume as described previously. The resulting [13C]bicarbonate and [1-13C]pyruvate signals were each reconstructed using an automatic off-resonance correction algorithm as described previously. For each set of [13C]bicarbonate and [1-13C]pyruvate images, the mean metabolite signal per unit volume was computed in the anterior half of the ventricular wall of each pig heart. For each measurement, the signal from the anterior myocardium (as chosen from the anatomical images) was summed, and this number was divided by the area of the region multiplied by the slice thickness. This was measured in the image with maximal [13C]bicarbonate signal. The resulting [13C]bicarbonate and [1-13C]pyruvate signals were each normalized to the mean [1-13C]pyruvate signal per unit volume within the LV chamber, also measured from the image with maximal [13C]pyruvate signal (i.e. the peak of the bolus). This gave ratio indices of PDC-mediated pyruvate oxidation and lactate production via lactate dehydrogenase (LDH).

Statistical analysis

Data are expressed as the mean ± SEM. A normal distribution was confirmed using a Kolmogorov–Smirnov test. For the longitudinal changes in physiological data and all data resulting from non-invasive MR experiments, repeated measures one-way analysis of variance (ANOVA), followed by a post-hoc paired, two-sided Student’s t-test with Bonferroni correction for multiple comparisons, was used. An unpaired, two-sided Student’s t-test was used for comparisons between DCM and control biochemistry data (n = 5 in each group). All statistical analyses used GraphPad Prism (GraphPad, La Jolla, CA, USA). Significant changes were considered for P < 0.05.

Results

Pacing-induced dilated cardiomyopathy

Pigs developed heart failure after 4.6 ± 0.5 weeks of RV pacing. Physiological parameters at baseline, and as pigs developed DCM, are shown in Table 1.

Cine-magnetic resonance imaging

Cine-MRI revealed substantial changes to LV and RV structure and function with the development of heart failure, as described in Figure 2 and in the Supplementary material, Table S1.

Phosphorus-31 magnetic resonance spectroscopy

A representative 31P spectrum, taken from a voxel placed in the in vivo pig heart 2 weeks after pacing, is shown in the Supplementary material, Figure S1. 31P MRS revealed that myocardial PCr/ATP was progressively depleted throughout the pacing protocol, from 2.3 ± 0.2 at baseline to 1.7 ± 0.1 at early pacing, and further to 1.3 ± 0.2 with moderate subclinical dysfunction (Supplementary material, Figure S1). In DCM, 31P MR spectra had a low signal-to-noise ratio to the extent that they were not reproducibly quantifiable, presumably due to the depletion of ATP in

| Table 1 | Physiological parameters with the development of heart failure |
|---------|------------------------------------------------------------|
|         | Baseline | Early          | Moderate       | DCM            |
| Heart rate, b.p.m. | 118 ± 9 | 89 ± 7*       | 85 ± 5*        | 86 ± 5*        |
| Body weight, kg    | 25 ± 2  | 30 ± 2        | 34 ± 2*        | 35 ± 3*        |
| Body surface area, m² | 0.60 ± 0.03 | 0.68 ± 0.02 | 0.74 ± 0.02*   | 0.76 ± 0.04*   |
| Creatinine, μmol/L | 106 ± 9 | 126 ± 17      | 160 ± 17       | 178 ± 23*      |
| Urea, mmol/L       | 4.6 ± 0.6| 5.0 ± 0.4     | 5.5 ± 0.5      | 5.9 ± 0.3*     |
| Cholesterol, mmol/L| 2.3 ± 0.2| 1.9 ± 0.2     | 1.9 ± 0.1      | 1.4 ± 0.3*     |
| Glucose, mmol/L    | 5.3 ± 0.9| 4.6 ± 0.5     | 4.8 ± 0.5      | 5.3 ± 0.8      |
| Insulin, pmol/L    | 57 ± 13 | 53 ± 8        | 47 ± 8         | 59 ± 9         |
| FFAs, mmol/L       | 0.11 ± 0.03| 0.16 ± 0.04 | 0.07 ± 0.03    | 0.07 ± 0.04    |
| Triglycerides, mmol/L | 0.30 ± 0.10 | 0.28 ± 0.06 | 0.30 ± 0.07    | 0.13 ± 0.06*   |

DCM, dilated cardiomyopathy; FFAs, free fatty acids.

*P < 0.05 compared with baseline.
pacing-induced DCM, measured here (see below) and previously, to decrease its MR signal.

**Hyperpolarized $^{13}$C magnetic resonance imaging and magnetic resonance spectroscopy**

**Hyperpolarized $^{13}$C magnetic resonance spectroscopy**
A representative MR spectrum is shown in Figure 3A, with peaks from the infused $[2-^{13}$C$]$pyruvate, as well as the metabolic products $[5-^{13}$C$]$glutamate (183 p.p.m.) and $[1-^{13}$C$]$acetyl carnitine (175 p.p.m., not quantifiable with moderate cardiac dysfunction and DCM). Peak assignments were made by comparison with previous $[2-^{13}$C$]$pyruvate studies performed ex vivo and in vivo in rats. The effects of early pacing, moderate dysfunction, and DCM on cardiac $[5-^{13}$C$]$glutamate production are shown in Figure 3. At the baseline time point, the maximum $[5-^{13}$C$]$glutamate/$[2-^{13}$C$]$pyruvate ratio was $4.3 \pm 0.9\%$. Early after the onset of pacing, the $[5-^{13}$C$]$glutamate/$[2-^{13}$C$]$pyruvate ratio was reduced by 51\%, to $2.1 \pm 0.6\%$, and stayed at this level throughout disease progression ($P < 0.05$).

As described previously and in Figure 3, the production of $[5-^{13}$C$]$glutamate from $[2-^{13}$C$]$pyruvate resulted from (i) PDC-mediated formation of $[1-^{13}$C$]$acetyl-CoA; (ii) $^{13}$C flux through the first span of the Krebs cycle, into mitochondrial $\alpha$-ketoglutarate; (iii) $^{13}$C flux through the oxoglutarate–malate carrier (OMC), instead of via the Krebs cycle enzyme $\alpha$-ketoglutarate dehydrogenase ($\alpha$KGDH); and finally (iv) conversion of $[\gamma-^{13}$C$]$ $\alpha$-ketoglutarate into $[5-^{13}$C$]$glutamate. The change to $[5-^{13}$C$]$glutamate production in the absence of altered PDC-mediated pyruvate oxidation (see below) indicated either that the relationship between Krebs cycle and OMC fluxes...
changed early after the onset of pacing, or that the glutamate pool size was depleted.

Hyperpolarized $^{13}$C magnetic resonance imaging

Figure 4A shows representative in vivo images of infused $[{^{1-13}}C]pyruvate$ and its metabolism into $[{^{13}}C]bicarbonate$ and $[{^{1-13}}C]lactate$. Visual inspection of images (shown in Figure 4A) indicated that less of the infused bolus of hyperpolarized $[{^{1-13}}C]pyruvate$ reached the left ventricle in hearts with DCM. Maximum $[{^{13}}C]bicarbonate$ signal production across the anterior wall of the myocardium was normalized to LV $[{^{1-13}}C]pyruvate$ to give a qualitative index of pyruvate oxidation by the PDC. At baseline, $[{^{1-13}}C]bicarbonate/[{^{1-13}}C]pyruvate$ ratio yielded a value of $0.016 \pm 0.002$, which remained constant through early pacing and with

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**Figure 3** Hyperpolarized $^{13}$C magnetic resonance spectroscopy (MRS) showing altered $[{^{5-13}}C]glutamate$ production during development of dilated cardiomyopathy (DCM), following infusion of hyperpolarized $[{^{2-13}}C]pyruvate$. (A) Representative spectra taken from a healthy pig (left), and from the same pig after 3 weeks of pacing, when it had moderate cardiac dysfunction. (B) Representative time courses of the infused $[{^{2-13}}C]pyruvate$ and its conversion into $[{^{5-13}}C]glutamate$, following quantification of the spectra shown in A. (C) The $[{^{5-13}}C]glutamate/[{^{2-13}}C]pyruvate$ ratio, measured for all pigs during development of DCM.
figure 4 hyperpolarized 13c magnetic resonance imaging (mri) results describing alterations to pyruvate dehydrogenase complex (pdc) flux and [13c]lactate production with the pathogenesis of dilated cardiomyopathy (dcm), following infusion of hyperpolarized [1-13c]pyruvate. (a) representative pyruvate (pyr, top), bicarbonate (bic, middle), and lactate (lac, bottom) 13c mri images taken from the same pig and at weekly intervals during the pacing protocol, until dcm developed. the images displayed for each metabolite were selected from the same, mid-papillary slice and in the same respiratory cycle. signal intensity in the pyruvate image was scaled based on 15–100% of the maximum pyruvate signal at week 0, whereas the bicarbonate and lactate signal intensities were scaled based on 15–100% of the maximum bicarbonate signal intensity at week 0. (b) relative changes to pdc flux with dcm in five pigs.

in vitro heart tissue analysis

after the development of dcm, myocardial atp levels as assessed by a luciferase assay decreased by 41%, from 34.2 ± 4.0 to 20.2 ± 2.2 pmol/μg protein. protein content of phosphorylated akt decreased by half. pyruvate dehydrogenase kinase 4 (pdk4), normalized to glyceraldehyde 3-phosphate dehydrogenase (gapdh) protein content, was increased by nearly two-fold (figure 5). additionally, expression of the genes encoding peroxisome proliferator-activated receptor-α (ppara) and its downstream target carnitine palmitoyltransferase-1 (cpt1; normalized to gapdh) was significantly lower in dcm by 50% and 32%, respectively.

importantly, expression of the gene encoding sarcolemmal pyruvate transporter mct1 was unchanged with dcm development. total protein contents of the e1α subunit of the pdc, ldh b, and glucose transporters 1 and 4 (glut1 and glut4) were also unchanged between healthy and failing hearts. phosphorylation of both as160 (akt substrate of 160 kda), and 5’ amp-activated protein kinase (ampk) was unchanged in dcm, with the latter observation further evidenced by the lack of...
change in genes encoding the AMPK downstream target acetyl-CoA carboxylase (ACC). Finally, gene expression of PGC1-α and PGC1-β was also unchanged in failing hearts.

**Discussion**

Here, we proved our initial hypothesis to be true: in vivo, in a clinically relevant large animal model, hyperpolarized $^{13}$C MR uncovered that the profile of pyruvate metabolism varied with the development of heart failure. Specifically, flux of pyruvate through the first span of the Krebs cycle and the OMC changed from baseline to the earliest stage of cardiomyopathy, and pyruvate oxidation by the PDC changed between moderate cardiomyopathy and the onset of DCM. After 1–2 weeks of rapid pacing, we observed reduced $[13C]$glutamate production from hyperpolarized $[2-^{13}C]$pyruvate; the physiological significance of this change was indicated by concomitant decreases in both cardiac index (CI) and energy reserve (PCr/ATP). Continued pacing into moderate dysfunction (2–5 weeks) did not alter pyruvate metabolism further, but decreased PCr/ATP and EF significantly. After the onset of heart failure (4–6 weeks), when clinical features of the disease were observed alongside LV wall thinning, dilatation, and further functional impairment, the in vivo bicarbonate/
[1-13C]pyruvate ratio decreased dramatically, suggesting reduced capacity for pyruvate oxidation by the PDC. PDK4 protein levels were increased, ATP levels were reduced, and activation of both Akt and PPARα was reduced. An overview of LV remodeling in DCM is shown in Figure 6.

### Early-onset metabolic perturbations

At an early stage of cardiomyopathy, 13C incorporation into the glutamate pool was reduced by 51%, the PCr/ATP ratio was reduced by 26%, and while CI was reduced by 25%, neither EF nor cardiac structure yet showed any change. The non-invasive nature of our study precluded collection of myocardial biopsies during DCM development, so validating the physiological mechanism altering [13C]glutamate production was outside the scope of this study. With further validation in future, however, these results could have two potential clinical applications: first, [13C]glutamate production could be an early diagnostic biomarker for aetologies of heart failure characterized by energetic depletion. Secondly, altered mitochondrial energy metabolism may have had a causal role in heart failure pathogenesis.

### Altered metabolism in dilated cardiomyopathy

Our results showed that in vivo, oxidation of [1-13C]pyruvate by the PDC was maintained throughout early and moderate cardiomyopathy. Additionally, in DCM, we measured reduced ATP content, and reduced expression of the genes encoding the regulator of fatty acid oxidation, PPARα, and its downstream target CPT1. These observations were consistent with many studies performed in patients and in experimental models, which have suggested that the failing heart is reliant on glucose metabolism due to decreased capacity for ATP generation via fatty acid oxidation.2,7,9–13

In DCM, however, hyperpolarized 13C MRI revealed a 67% reduction to in vivo oxidation of [1-13C]pyruvate by the PDC. This agrees with a study performed in cardiomyopathic hamsters showing reduced PDC activity.24 Furthermore, because hyperpolarized 13C MRI enabled serial measurements throughout disease pathogenesis, this is the first study to identify a temporal association between altered cardiac PDC flux and the transition to decompensated heart failure.2 Our data suggest that failing hearts compensated throughout early and moderate pacing via an increased reliance on carbohydrate oxidation via the PDC to produce ATP, depleting the energetic reserve (PCr/ATP) without major detriment to contractility or geometry (Figure 6). Ultimately, hearts may have failed when they lost the ability to use pyruvate as fuel to generate ATP in the context of already reduced fatty acid oxidation capacity.

Biochemical analyses helped to clarify the mechanisms contributing to decreased in vivo cardiac PDC flux. Though total expression of the E1α subunit of the PDC was unchanged in DCM, protein expression of PDK4, which phosphorylates and inhibits PDC, was elevated by 94%. Therefore, we expect that PDK4-mediated inhibition contributed to the reduced in vivo [13C]bicarbonate production from PDC.

Dilated cardiomyopathy decreased Akt phosphorylation by 54%. This finding agreed with a study by Nikolaidis et al., which confirmed that Akt phosphorylation was reduced due to increased phosphatase and tensin homologue (PTEN) phosphatase expression in tachycardia-induced DCM, resulting in impaired GLUT4 translocation to the sarcolemma, defective myocardial insulin signaling, and whole-body insulin resistance.16 As insulin directly activates PDC via protein kinase C-δ and pyruvate dehydrogenase phosphatase (PDP),28 defective myocardial insulin signalling may have also contributed to the reduction in PDC-mediated [1-13C]pyruvate oxidation that we observed.

This study has revealed the importance of serially assessing pyruvate metabolism under in vivo conditions, at frequent time points as heart failure progresses. We saw that the capacity for PDC to oxidize pyruvate changed dramatically over just 1 week, with no contribution from anaplerosis, as the heart transitioned from compensated cardiomyopathy to decompensated DCM. Studies not performed serially throughout DCM development, that simply compared metabolism in healthy subjects and subjects with severe heart failure, may have missed the onset of this change and thus the association between PDC capacity and DCM. Furthermore, studies reporting increased glucose uptake in DCM do not necessarily imply increased glucose oxidation, as PDC activity must also be high for efficient use of glucose as energetic fuel. Our in vivo hyperpolarized [1-13C]pyruvate oxidation measurements suggest that treatments that ameliorate insulin resistance (glucagon-like peptide-1 agonists15) or block FFA uptake (carnitine palmitoyltransferase-I inhibitors39) improve myocardial energetics because they also enhance flux through PDC.

### Study limitations

One limitation of this study was that we did not take myocardial tissue samples at each experimental time point. Future biochemical investigation of metabolic alterations occurring after 1–2 weeks of pacing is warranted to determine the mechanism driving reduced [13C]glutamate production. If we can validate this mechanism, in future it may be possible to use [13C]glutamate production as a biomarker to diagnose cardiomyopathy and to optimize its treatment in the clinic.

A second limitation was that the delivery of hyperpolarized [1-13C]pyruvate to the myocardium and its uptake into cardiomyocytes could have influenced our measurements. Accordingly, we accounted for changes to myocardial blood flow throughout DCM development by dynamically imaging the infused hyperpolarized [1-13C]pyruvate bolus, and normalizing metabolite signals to the LV signal from infused [1-13C]pyruvate. To our knowledge, no changes to basal myocardial perfusion have been reported in tachycardia-induced DCM. Further, mRNA encoding the monocarboxylate transporter (MCT,40 which enables pyruvate uptake into cardiomyocytes) did not change in DCM, suggesting that [1-13C]pyruvate uptake also remained constant. However, MCT expression increased in heart failure following myocardial infarction41 indicating that in other disease aetiologies hyperpolarized 13C MR may overestimate carbohydrate oxidation. In future, this issue can be avoided by using dual-labelled [1, 2-13C]pyruvate to monitor [13C]glutamate and [13C]bicarbonate production simultaneously.42 This approach would enable measurement of a [13C]glutamate/[13C]bicarbonate ratio that only considers 13C labels reaching the cardiac mitochondria (illustrated in Supplementary material, Figure S2), and would be insensitive to factors such as...


[13C]pyruvate delivery, uptake, and blood pool size due to ventricular dilatation.

Significance of this work

Hyperpolarized [1-13C]pyruvate was administered to patients for the first time in 2010, with a view towards using metabolic MRI to characterize prostate cancer. The study presented here offers the first evidence that using hyperpolarized 13C MR to follow cardiovascular disease progression in patients is also feasible. We applied novel hyperpolarized 13C MRI and MRS data acquisition and analysis methods that are promising for direct translation into humans for the following reasons: (i) the dose of [13C]pyruvate tracer was half of the dose that has been used in the first patient study; (ii) we acquired cardiac-gated 13C MRI and MRS data from free-breathing pigs on a standard whole-body MR scanner, using a sequence that could easily be implemented in free-breathing patients; (iii) dynamic imaging of the [1-13C]pyruvate input bolus enabled ratiometric data analysis, which accounted for the altered tracer pharmacokinetics that occur with disease; and (iv) each 13C scan was completed within ~2 min, considerably faster than 31P MRS, breath-hold cine-MRI, and PET scans can practically be executed in people.

Moreover, the results of this study illustrate how hyperpolarized 13C MRS/MRI could be useful to diagnose heart failure and to optimize its treatment.25,30 We identified distinct profiles of substrate utilization based on markers acquired non-invasively with hyperpolarized 13C MR (i.e. normal, reduced [13C]glutamate production only, and both reduced [13C]glutamate and [13C]bicarbonate production). In future, other distinct profiles of 13C-labelled tracer metabolism may emerge that correlate with cardiomyopathy stage and aetiology. The concept of metabolic stress testing, in which dobutamine stress is used to assess the heart’s potential to increase carbohydrate oxidation at the level of the PDC to increase ATP production subsequently, could also play a role to identify stage-specific changes to metabolic flexibility. If this is the case, metabolic profiling with hyperpolarized 13C MR may be useful for clinicians to select which patients could benefit from treatment with pharmacological agents that modulate metabolism, and, furthermore, may suggest which metabolic pathway may be the best to target (i.e. trimetazidine and carnitine palmitoyltransferase-1 inhibitors to limit fatty acid oxidation, or glucagon-like peptide-1 agonists to improve insulin sensitivity). Serial 13C metabolic profiling may also be useful to monitor the efficacy of heart failure treatments, particularly metabolic modulators, but also including non-metabolic drugs and interventional therapies that improve cardiac efficiency.5,6

Conclusions

In summary, by applying hyperpolarized 13C MRI and MRS to an experimental model of heart failure, we performed the first non-invasive measurements of cardiac pyruvate metabolism throughout disease pathogenesis, alongside measurement of myocardial energetics, structure, and function. At an early stage of disease, hyperpolarized 13C MRS revealed an alteration to [13C]glutamate production, concomitant with impaired cardiac function and energetics. Further, by using hyperpolarized 13C MRI to measure [1-13C]pyruvate oxidation by the PDC serially at frequent time points, we identified a temporal association between reduced pyruvate oxidation and the onset of overt heart failure. With the use of methods such as those presented here, it is possible that metabolic MR with hyperpolarized 13C-labelled tracers will form an important part of both basic cardiovascular research and routine clinical diagnosis and treatment monitoring in cardiology.

Supplementary material

Supplementary material is available at European Journal of Heart Failure online.

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Conflict of interest: A.P.C. is an employee of GE Healthcare. This work received research support from Medtronic and GE Healthcare. G.A.W holds stock in GE Healthcare, the principal commercial developers of hyperpolarized 13C technology, and is currently conducting research sponsored by this company. He has also received a speaker’s honorarium from GE Healthcare within the past 12 months. C.H.C. receives research support from GE Healthcare who own many patents on DNP technology. D.J.T. has previously received equipment support from GE Healthcare and Oxford instruments. All other authors have no conflicts to declare.

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