Dynamic Nuclear Polarization and MRI for the study of cardiac metabolism

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Abstract

Dynamic Nuclear Polarization (DNP) is an emerging technique in magnetic resonance imaging (MRI) that is able to enhance or "hyperpolarize" the signal of ^{13}C by many orders of magnitude in a wide range of endogenous metabolites. This enables us to image not only an injected parent molecule such as pyruvate but also its metabolic fate due to enzymatic conversion. The technology is being used for kinetic studies in solutions of viable cells, ex-vivo in perfused organs, in-vivo in preclinical disease models and now with a successful phase I clinical trial there is the potential to translate these technologies into the clinic. DNP has great potential for the study of altered cardiac metabolism under pathophysiological conditions.

Keywords: dynamic nuclear polarization; MRI; cardiac metabolism; ^{13}C.

■ Heart Metab. (2012) 55:13–17

Introduction

The heart consumes and recycles approximately 6 kg of adenosine triphosphate (ATP) every day, deriving energy for contraction and ionic homeostasis. Most of this extraordinary energy demand is supplied by continuous mitochondrial oxidation of fatty acids and pyruvate derived from glycolysis [1]. It would be highly desirable to be able to non-invasively visualise and quantify these metabolic processes, to provide new insight into how these change in disease. Access to such information would be invaluable for diagnosis, prognosis, and assessment of response to therapy. Cardiovascular magnetic resonance (MR) is a powerful non-invasive technique to assess cardiac morphology and function and to distinguish regions of infarct from viable tissue of myocardium. Magnetic resonance spectroscopy (MRS) employing ^{31}P can be used to measure intracardiac concentrations of ATP and phosphocreatine [2], while ^{1}H can be used to measure triglyceride accumulation in ischemic regions [3]. With higher sensitivity 3-tesla (3T) clinical MR systems becoming more widely available, both ^{31}P and ^{1}H MRS have been extended to detect metabolic changes in patients affected by aortic stenosis, cardiomyopathy, ischemia and diabetes [4–8]. Despite the advances, the limited sensitivity of MRS leads to long scan times, and hence only steady-state metabolite concentrations can be measured.

The recently introduced concept of dissolution dynamic nuclear polarization (DNP) provides a method to enhance the MRS signal and thus the MR sensitivity of biologically important nuclei such as ^{13}C or ^{15}N by greater than a factor ten thousand [9]. Using this technique, injectable hyperpolarized molecules can be used to probe metabolism *in vivo* [10]. For example, pyruvate is situated at a metabolic crossroads between glycolysis and oxidative phosphorylation (Fig. 1), and in combination with DNP, is proving a valuable tool in the study of metabolism in cancer [11] and in cardiovascular disease [12]. Pyruvate metabolism in the heart is largely driven by high activity of the mitochondrial pyruvate dehydrogenase (PDH) complex, under tight phosphorylation-mediated regulation by pyruvate dehydrogenase kinase (PDK) and pyruvate dehydrogenase phosphatase (PDP). Pyruvate has various competing fates, either entering the TCA cycle for catabolism, anaplerotic conversion to oxaloacetate by pyruvate carboxylase, transamination to form alanine, or under ischemic conditions or mitochondrial dysfunction, to the production of lactate by lactate dehydrogenase (LDH). The interplay of fatty acid β oxidation vs pyruvate oxidation is controlled by the Randle cycle, and is sensitive to up- or down-regulation of fatty acid and glucose transport, to changes in PDH activity, the availability of enzyme cofactors such as NADH/NAD+, and endogenous concentrations of metabolic precursors or products. Modulations in glucose metabolism arising from nutritional status, response to insulin in diabetes, lipid overload due to obesity or changes in mitochondrial function due to ischemia lead to significant shifts in metabolism between fatty acid oxidation and anaerobic glycolysis [13]. Conditions such as cardiac hypertrophy lead to metabolic remodeling, upregulated glucose metabolism, increased activity of lactate dehydrogenase (LDH) and excretion of lactate. Unchecked, these metabolic alterations result in exhaustion of intracellular ATP, acidosis, loss of ionic homeostasis, and apoptosis or necrosis [14].

Dynamic nuclear polarization and hyperpolarized MRI

Nuclei with spin-quantum number I = 1/2 (e.g. ^{1}H and ^{13}C) possess a magnetic moment that can orient either parallel or antiparallel when placed in an external magnetic field. Hyperpolarization aims to greatly enhance the population difference or polarization and thereby boost MR sensitivity by many orders of magnitude (Fig. 2). The technique utilizes the high polarization of unpaired electrons at very low temperatures. Samples isotopically enriched in ^{13}C or ^{15}N are dissolved in a glass forming solvent containing a low-concentration

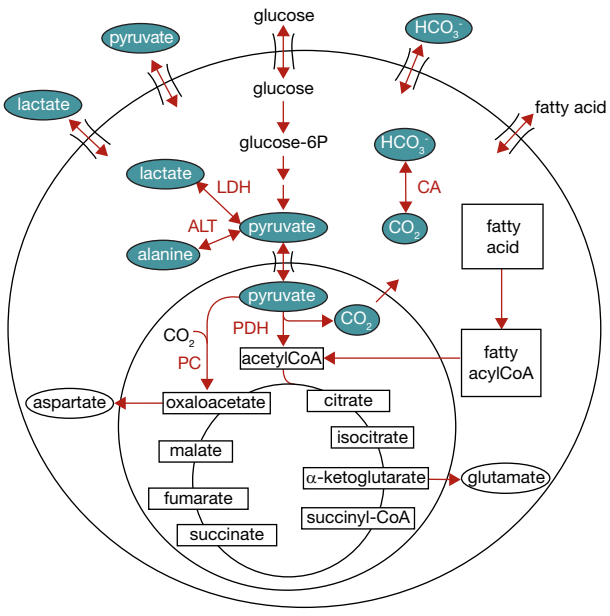

Fig. 1 *Schematic diagram of key metabolic reactions in the heart and some of the pathways that can be interrogated using hyperpolarized pyruvate labeled in the C1 position (blue).*

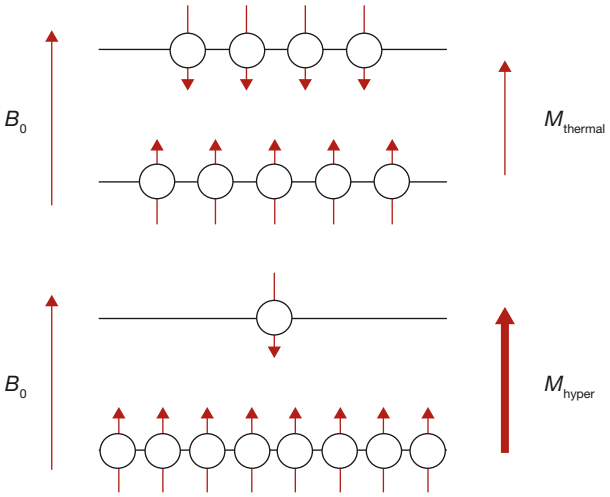

Fig. 2 *The sensitivity of MRI is determined by the very small population difference between nuclear spin states when placed in a strong magnetic field B_0 (top). This population difference is typically of the order 10^{-6} or parts per million making MRI an inherently insensitive technique. Hyperpolarization techniques aim to significantly boost this small thermal population difference by many orders of magnitude (bottom) and thereby dramatically enhance MR sensitivity.*

of a free radical. They are then frozen in liquid helium within a superconducting magnet (B_0 = 3.35T) and pumped under vacuum to reach temperatures of the order 1.2-1.4K. Electron polarization is transferred to nuclear polarization under the action of a microwave field, and typically takes of the order of 1-2 hours to polarize a sample. The frozen substrate is rapidly melted and dissolved in a volume of hot physiological buffer to yield a biologically compatible solution retaining its hyperpolarized signal. Fig. 3 shows a typical time-series of ^{13}C spectra acquired for a 50mM solution of hyperpolarized [1-^{13}C] pyruvate, and a single acquisition per spectrum acquired on a 9.4T spectrometer. The signal decay for [1-^{13}C] pyruvate at this field strength, governed by the longitudinal ^{13}C relaxation time (T_1) is ~54s, and is typically in the range 20-60s for most molecules of biological interest. The primary limitation of the technique is the decay of the hyperpolarized signal back to thermal polarization. Nonetheless the time-window is sufficient to carry out real-time measurements following injection of the hyperpolarized solution into biological systems.

The perfused heart—a pharmacological tool

Metabolism can be studied in Langendorff perfused hearts with a range of hyperpolarized substrates in real-time, and mathematical models can be developed to estimate enzyme kinetics. Injection of hyperpolarized [1-^{13}C] pyruvate into the perfused rat heart [15–16], figure 4, leads to conversion of pyruvate into lactate, or alanine, or to the production of CO_2 via PDH, which is in turn converted to bicarbonate by carbonic anhydrase. This exchange of bicarbonate and CO_2 is in pH-dependent equilibrium, and their relative ratios can be used to calculate myocardial pH [17]. The presence of the soluble fatty acid octanoate [15] leads to significant reduction in the conversion to bicarbonate and increased conversion to lactate, reflecting the metabolic switch between fatty acid and pyruvate oxidation. Likewise, global ischemia leads to increased lactate production and decreased PDH flux measured with [1-^{13}C] pyruvate [16], to decreased citrate and glutamate measured with [2-^{13}C] pyruvate [18], and to changes in carnitine buffering measured with hyperpolarized [1-^{13}C] acetate and [1-^{13}C] propionate [19], reflecting reduced TCA cycle oxidation and increased anaerobic metabolism.

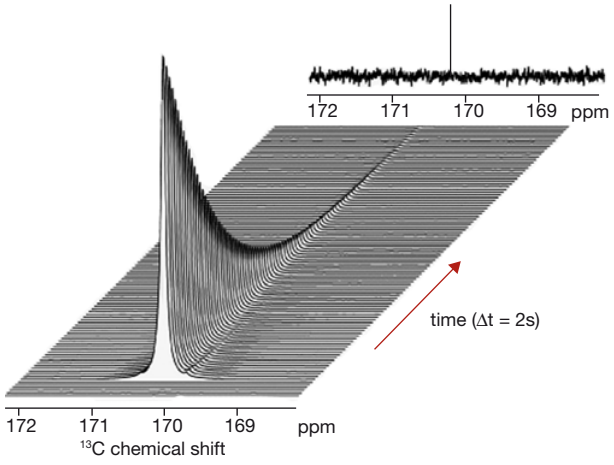

Fig. 3 Time series of hyperpolarized ^{13}C spectra acquired for a 50mM solution of [1-^{13}C] pyruvate acquired with a small flip angle pulse (1deg) and a temporal resolution of Δt = 2s. Top shows the thermal ^{13}C signal acquired for the same sample after the signal has decayed, acquired with co-addition of 32 scans. The difference in peak areas between the first hyperpolarized spectrum and the thermal signal is about 15,000 fold in this example.

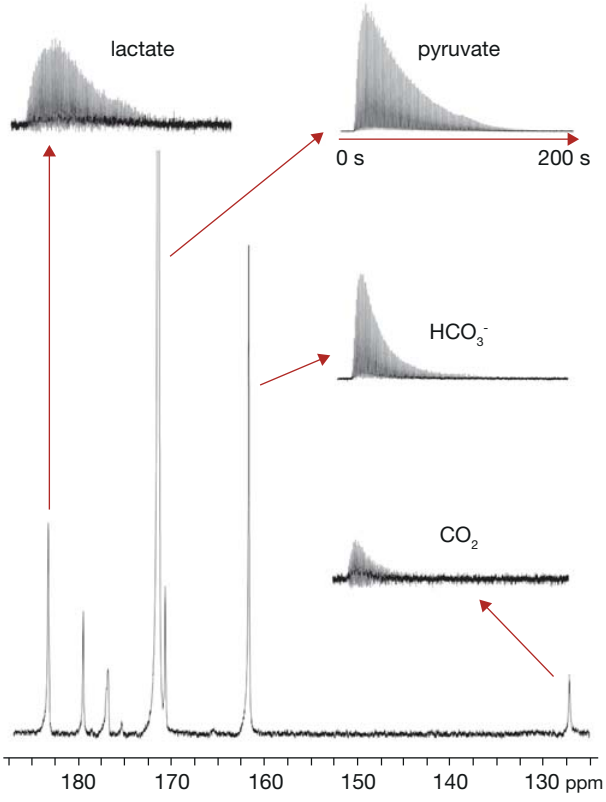

Fig. 4 ^{13}C spectrum acquired following injection of a solution of hyperpolarized [1-^{13}C] pyruvate into a Langendorff perfused rat heart. The injected pyruvate peak is clearly seen as well as a number of metabolites, including CO_2, bicarbonate, lactate and alanine. Temporal variations of each metabolite peak are measured and the time dependences correlate with the respective enzyme activities in the heart. Reprinted from Merritt et al [16] with permission of the publisher. Copyright © John Wiley and Sons.

Cardiac metabolism *in vivo*

Experiments can be extended *in vivo* through intravenous injection of hyperpolarized solutions. The *in vivo* rates of PDH flux in rats measured with hyperpolarized [1-^{13}C] pyruvate, correlate with PDH activity measured by enzymatic assay [20], demonstrating marked reductions in PDH flux following overnight starvation and in streptozotocin (STZ) induced type I diabetic rats [21]. Decreased PDH flux was also observed in response to triiodothyronine (T3) induced hyperthyroidism and hypertrophy, with increased expression of PDK4, decreased PDH flux as well as increased conversion to lactate via LDH [22]. These metabolic changes were renormalized on treatment with the PDK inhibitor dichloroacetate (DCA).

Non-spatially selective spectroscopy is sufficient to estimate global metabolic changes *in vivo*; however, to distinguish regional changes, spatially resolved measurements employing ^{13}C chemical shift imaging are required. The approach was first demonstrated in the pig heart [23] where it was possible to observe clear regional reductions in pyruvate to bicarbonate conversion following 15min or 45min coronary occlusion, and at the later time-point correlated with a region of infarct observed in the late Gd enhanced images. Using newly developed MR sequences and improvements in hardware, imaging gradient and coil design, it is now possible to acquire rapid multi-slice cardiac-gated ^{13}C images in the pig heart using spectral-spatial excitation pulses combined with single-shot spiral readout for rapid whole-heart imaging [24] (Figs. 4, 5).

Clinical translation

The instrumentation required to carry out DNP is largely experimental or home-built apparatus and currently not suitable for clinical use. A phase I first in man study has been carried out in a collaboration between GE Healthcare and UCSF using modified hardware to carry out the DNP process under FDA approval within a clean room and incorporating quality assurance steps to remove the free radical and to verify parameters such as solution temperature and pH. This first trial has been completed in 31 men with confirmed prostate cancer and metabolic imaging achieved using [1-^{13}C] pyruvate [25]. Initial experience is very encouraging for further development of the technique. Commercialization of a DNP polarizer for sterile use intent [26] will enable the technology to be expanded to other centers for further phase I studies and validation of the technology. We await the first demonstration of DNP applied to cardiac studies in humans and the next few years promise an exciting period in the development of these novel technologies.

Conclusion

DNP shows enormous potential for the study of metabolic changes associated with cardiac disease in both the laboratory setting for basic research but also in a clinical setting. Progress over the coming years will be driven on the one hand by technological advances and overcoming the necessary regulatory approvals, but also by the demands or unmet needs of cardiologists and oncologists in the stratification of disease or in the

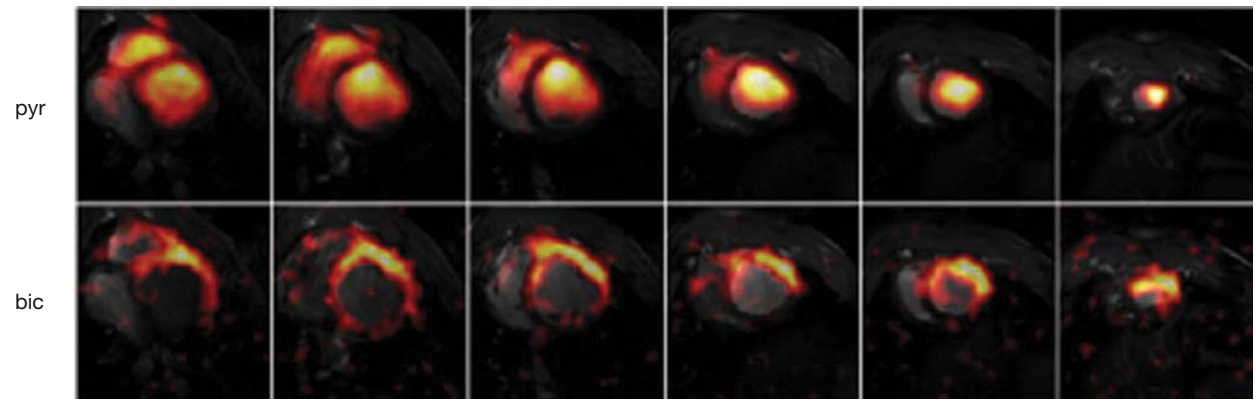

Fig. 5 *Multi-slice cardiac-gated ^{13}C images with 8.8mm in-plane resolution and a 1cm slice thickness in a healthy pig heart using spectral-spatial excitation pulses combined with single-shot spiral readout. The top row shows metabolic images of the injected pyruvate overlaid on the conventional anatomical MR images whilst the bottom row shows that of bicarbonate arising from enzymatic conversion. Reprinted from Lau et al [24] with permission of the publisher. Copyright © John Wiley and Sons*

development of novel therapies. DNP is uniquely placed to interrogate the biochemical changes that influence the balance between glucose or fatty acid oxidation and anaerobic metabolism in the healthy or failing heart. •

Acknowledgements Wellcome Trust Biomedical Engineering Centre and The Royal Society.

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