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REVIEW

Biomedical Applications of the Dynamic Nuclear Polarization and Parahydrogen Induced Polarization Techniques for Hyperpolarized ^{13}C MR Imaging

Neil J. Stewart and Shingo Matsumoto*

Since the first pioneering report of hyperpolarized $[1-^{13}\text{C}]$ pyruvate magnetic resonance imaging (MRI) of the Warburg effect in prostate cancer patients, clinical dissemination of the technique has been rapid; close to 10 sites worldwide now possess a polarizer fit for the clinic, and more than 30 clinical trials, predominantly for oncological applications, are already registered on the US and European clinical trials databases. Hyperpolarized ^{13}C probes to study pathophysiological processes beyond the Warburg effect, including tri-carboxylic acid cycle metabolism, intra-cellular pH and cellular necrosis have also been demonstrated in the preclinical arena and are pending clinical translation, and the simultaneous injection of multiple co-polarized agents is opening the door to high-sensitivity, multi-functional molecular MRI with a single dose. Here, we review the biomedical applications to date of the two polarization methods that have been used for *in vivo* hyperpolarized ^{13}C molecular MRI; namely, dissolution dynamic nuclear polarization and parahydrogen-induced polarization. The basic concept of hyperpolarization and the fundamental theory underpinning these two key ^{13}C hyperpolarization methods, along with recent technological advances that have facilitated biomedical realization, are also covered.

Keywords: ^{13}C metabolic MRI, dynamic nuclear polarization, hyperpolarization, molecular imaging, parahydrogen-induced polarization

Introduction

Hyperpolarization refers to a class of methods that enable the fundamental sensitivity limits of magnetic resonance imaging (MRI) to be overcome, allowing functional imaging of exogenous agents of unprecedented quality.¹ Over the last 20 years, hyperpolarized (HP) ^3He and ^{129}Xe noble gases have been developed from experimental tools into safe, inhalable contrast agents for high-resolution, functional MRI of the lung airspaces and are already used routinely in a clinical setting.² On the other hand, HP ^{13}C -labelled liquid-phase probes for molecular and metabolic MRI hold great promise for interrogating pathophysiology at the cellular level.³

The first *in-man* hyperpolarized $[1-^{13}\text{C}]$ pyruvate MRI exams in patients with prostate cancer realized the potential for observing metabolic processes beyond glycolysis, which is typically probed by ^{18}F -fluorodeoxyglucose positron-emission tomography (^{18}F -FDG-PET); until recently the only metabolic imaging method used routinely in the oncology clinic.⁴ This pioneering study has been followed by a rapid dissemination of HP $[1-^{13}\text{C}]$ pyruvate MRI for clinical applications,⁵ facilitated by the development of commercial, sterile polarization systems for clinical use.⁶ As of June 2019, more than 30 clinical trials worldwide pertaining to HP $[1-^{13}\text{C}]$ pyruvate MRI are either in a complete, in progress, or pending phase and this number is predicted to only increase further over the coming years.

In this review article, we provide a brief overview of the concept of hyperpolarization and the theory behind the methods to obtain liquid-state ^{13}C polarization; namely, dissolution dynamic nuclear polarization (d-DNP) and parahydrogen-induced polarization (PHIP), followed by a comprehensive review of the biomedical applications of HP ^{13}C MRI by, with a particular focus on recent clinical MRI applications of HP $[1-^{13}\text{C}]$ pyruvate and other hyperpolarized ^{13}C molecular imaging probes with clinical promise.

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Theoretical Background

Hyperpolarization

When placed in a magnetic field B_0 , spin- $\frac{1}{2}$ nuclei of gyromagnetic ratio γ occupy one of two Zeeman states at energies $\pm\gamma\hbar B_0/2$. The nuclear spin “polarization” is defined as the fractional difference in the population of the two states, which under conditions of thermal equilibrium is derived from the Boltzmann distribution:

$$P = \tan h \left(\frac{\Delta E}{2k_B T} \right) \approx \left(\frac{\gamma\hbar B_0}{2k_B T} \right) \quad (1)$$

The Boltzmann (thermal) polarization of ^{13}C —the only stable spin- $\frac{1}{2}$ carbon nucleus—at typical clinical magnetic field strengths is $\sim 10^{-6}$. In fact, MR of endogenous ^{13}C is challenging not just due to its \sim fourfold lower gyromagnetic ratio than ^1H ; the natural abundance of ^{13}C is only 1.1% and thus sensitivity is poor. A ~ 100 -fold MR signal enhancement can be obtained on endogenous tracers through ^{13}C -labeling, and a further 4–5 orders of magnitude enhancement via hyperpolarization.

Hyperpolarization denotes a temporary state of dramatic population excess in one nuclear spin state (see Fig. 1) and can be realized by a number of approaches; brute force polarization (utilizing low temperatures and high magnetic fields to directly increase the nuclear polarization)^{7,8}; spin-exchange optical pumping⁹ and metastability-exchange optical pumping¹⁰ for hyperpolarized gases; and d-DNP¹¹ and PHIP¹² for solution-state ^{13}C applications. The latter two methods have been demonstrated for biomedical ^{13}C molecular MRI

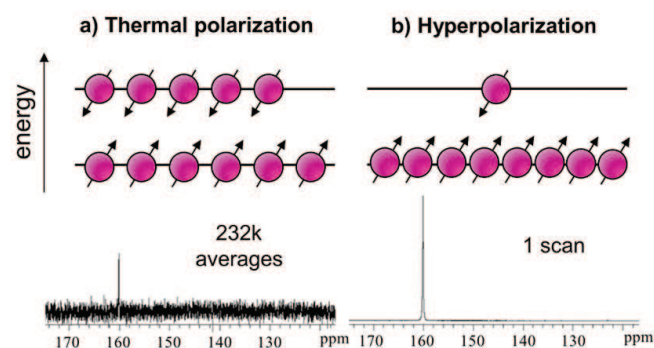


Fig. 1 Concept of hyperpolarization. (a) The occupation of nuclear Zeeman states of a spin- $\frac{1}{2}$ system in thermal equilibrium in a magnetic field follows that of the Boltzmann distribution [cf. Equation (1)]; for ^{13}C at 1.5T and 300 K, the polarization, i.e. the population difference between the spin up and down states for ^{13}C is only $P \sim 10^{-6}$. (b) Hyperpolarization describes the state of a large excess population in one of the nuclear Zeeman states, leading to a nuclear polarization several orders of magnitude greater than the Boltzmann polarization (Data is reproduced from the original dissolution dynamic nuclear polarization (d-DNP) paper¹¹ (Copyright (2003) National Academy of Sciences, USA) and compares NMR spectra obtained from thermally-polarized and hyperpolarized ^{13}C urea of ~ 60 mM concentration).

applications and these form the focus of this review article. We note that signal amplification by reversible exchange (SABRE),¹³ closely-related to conventional PHIP, is recently showing progress toward potential *in vivo* application¹⁴ but will not be covered in this article as biomedical application is yet to be shown; we refer the reader to Robertson and Mewis¹⁵ for an up-to-date review.

The MR signal enhancement associated with hyperpolarization is not permanent; longitudinal relaxation acts to return the nuclear spin state populations to that of thermal equilibrium, and after radiofrequency excitation, the hyperpolarized state is not recovered.¹⁶ Research into generating so-called “long-lived” states and also generation of continuously re-hyperpolarization¹⁷ are an active fields¹⁸; however, hyperpolarized $[1-^{13}\text{C}]$ pyruvate, the most promising molecule for clinical applications, remains limited by a $T_1 \sim 60$ s. The decay in magnetization associated with a number of RF excitations n with repetition time TR and flip angle α can be described as follows:

$$M_{xy}(n) = M_0 \exp \left[-(n-1) \frac{TR}{T_1} \right] \sin(\alpha_n) \prod_{j=1}^{n-1} \cos(\alpha_j) \quad (2)$$

For a constant flip angle, and $TR \ll T_1$, Equation (2) can be simplified to $M_{xy}(n) = M_0 \sin(\alpha) \cos^{n-1}(\alpha)$ [for example, after $N = 128$ RF excitations at flip angle 8° a magnetization of only $M_{xy}(N) \approx 0.3M_0 \sin(8^\circ)$ remains]. The signal decay during acquisition leads to filtering of the k -space and image blurring, but which can be somewhat compensated for by modifying the flip angle throughout the acquisition process.^{16,19} Nevertheless, acquisition of hyperpolarized signals necessitates efficient encoding of k -space, such as with spiral trajectories,²⁰ parallel imaging²¹ or compressed sensing.²² Hyperpolarized ^{13}C metabolic MRI relies upon the discrimination of MR signals from the injected probe (e.g. pyruvate) and its metabolic products (e.g. lactate) by chemical shift. If spatial information is not essential, dynamic spectroscopy is a simple and robust means to probe metabolism dynamics.²³ Several imaging strategies have been developed²⁴ including: phase-encoded chemical shift imaging (CSI)²⁵ which although inefficient, allows acquisition of full spectra; echo planar spectroscopic imaging, in which (usually fly-back) gradients are used for simultaneous 1D spatial encoding and spectral readout, permitting several-fold acceleration at the expense of SNR;^{26,27} spiral chemical shift imaging, wherein multi-dimensional spatial data is encoded simultaneously with spectral data in a similar manner to tomosynthesis;²⁸ spiral encoding schemes²⁹ combined with the robust iterative decomposition with echo asymmetry and least-squares estimation technique;³⁰ and spectral-spatial excitation for additional efficiency and the flexibility of a different flip angle on each resonance of interest.³¹ In light of the long T_2 of ^{13}C *in vivo*, SNR benefits have been realized by using single or multi-echo balanced steady-state free precession.^{32,33}

Dynamic nuclear polarization

Dissolution dynamic nuclear polarization—to date the principal polarization techniques employed to generate hyperpolarized $[1-^{13}\text{C}]$ pyruvate—relies upon the relatively large electron gyromagnetic ratio ($\gamma_e \approx 660\gamma_p$) which [according to Equation (1)] leads to an electron Boltzmann polarization of approximately unity at temperatures ~ 1 K at high field (see Fig. 2a).³⁴ An efficient electron paramagnetic agent (free radical, see e.g. Lumata et al.³⁵) is mixed with a glassing agent and the target probe to be polarized (e.g. pyruvate), which is cooled to ~ 1 K under a magnetic field of several tesla. In the subsequent glassy solid state where d-DNP is most efficient, microwave irradiation is used to induce polarization transfer from free electrons to ^{13}C nuclei over the course of ~ 1 h. At temperatures < 4.2 K, polarization transfer is believed to be primarily driven by the thermal mixing effect,³⁶ though depending on exact experimental conditions, contributions from the so-called solid effect and cross effect,³⁷ and the Overhauser effect in the solution phase,³⁸ may not be ignored. After polarization transfer, the frozen

sample is rapidly dissolved in a superheated solvent and transferred to the MRI system for measurement [hence the term “dissolution (d)”¹¹].

The first commercial d-DNP system for preclinical research applications shortly followed the publication of the original d-DNP paper¹¹ (HyperSense, Oxford Instruments, UK) and other efficient research systems have since been developed.³⁹ Most d-DNP systems including the HyperSense require large quantities of liquid helium to maintain the low sample temperature; however, two recent landmark developments have enabled d-DNP without consumption of cryogenics; a high-throughput, sterile polarizer for clinical applications SpinLab⁶ (GE Healthcare, Waukesha, WI, USA), and an efficient research polarizer with variable magnetic field (the SpinAligner, (Polarize, Frederiksberg, Denmark)),⁴⁰ both of which are commercially available. The SpinLab (Fig. 2a), operating at ~ 0.9 K and 5T and routinely obtaining up to 40% $[1-^{13}\text{C}]$ pyruvate polarization, is the only system to date approved for human application.

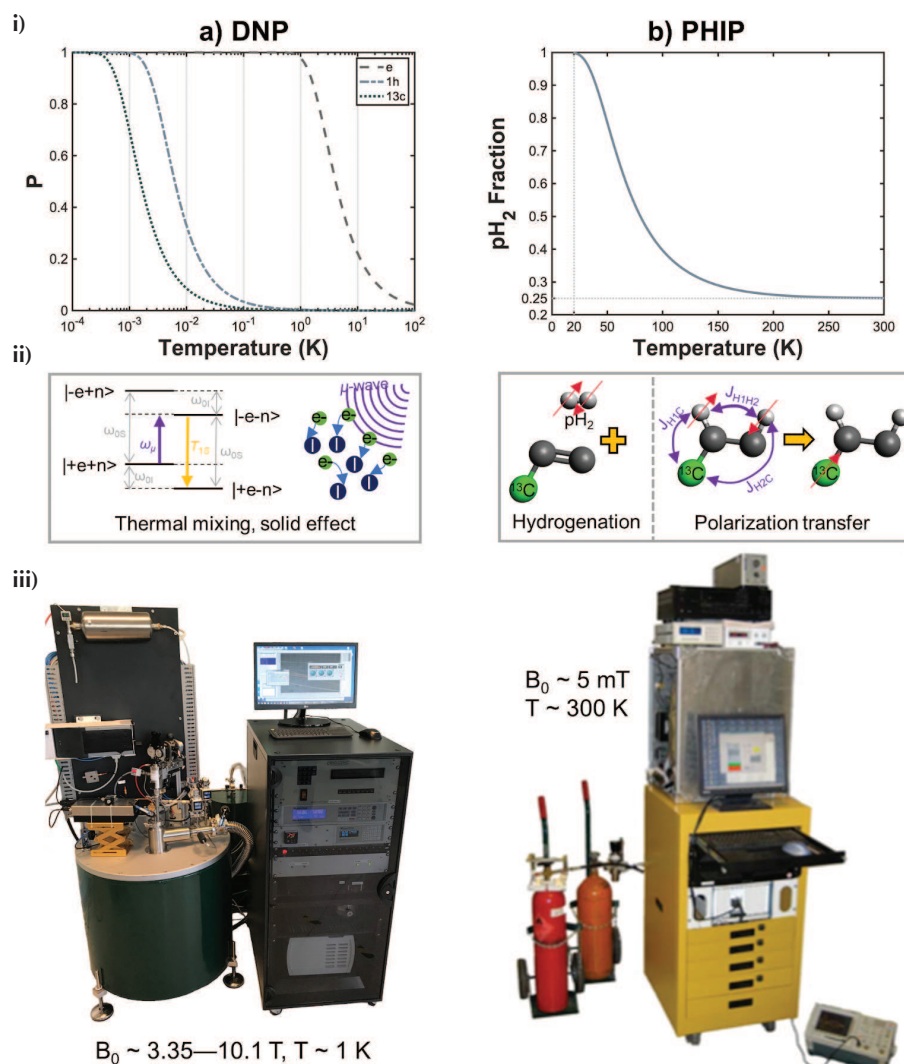


Fig. 2 Concept diagram for dissolution dynamic nuclear polarization (d-DNP) and parahydrogen-induced polarization (PHIP) polarization techniques. **(a)** In d-DNP, the source of ^{13}C nuclear polarization (P) is the approximately unity electron polarization (P) at low temperature and high magnetic field (curves plotted for 3.35T) (i). This is transferred to ^{13}C via microwave excitation (ii), predominantly mediated via the thermal mixing effect. (iii) Prototype commercial cryogen-free d-DNP system reported in Ardenkjaer-Larsen et al.⁴⁰ (original photo courtesy of Jan Henrik Ardenkjaer-Larsen, Technical University of Denmark and GE Healthcare). **(b)** In PHIP, the source of ^{13}C polarization is the inherent spin order of the parahydrogen polarization (P) at very high purity by cooling normal hydrogen in the presence of a paramagnetic catalyst (i). Parahydrogen is reacted with an unsaturated substrate, generating ^1H hyperpolarization, which is subsequently transferred to ^{13}C or other target heteronucleus (ii). Several dedicated low-field (mT) polarization systems have been designed for automating the hydrogenation and polarization transfer processes; the example shown is reprinted with permission from Springer Nature (Hövenner et al.).⁵⁸

Parahydrogen-induced polarization

Despite surmounting the hurdle associated with cryogen consumption, the initial outlay required for d-DNP systems remains high (~several million USD for the SpinLab). PHIP¹² is a relatively recent technique that offers a cheaper route to hyperpolarized ¹³C molecules for biomedical MRI applications.⁴¹ PHIP relies on the inherent spin order of parahydrogen, a spin isomer of hydrogen. At room temperature, the two spin- $\frac{1}{2}$ nuclei of each hydrogen molecule have an equal probability to occupy one of four spin states; three states of total spin 1 (ortho-hydrogen, “triplet” state) and one state of total spin 0 (parahydrogen, “singlet” state). When cooled in the presence of a paramagnetic catalyst (typically iron(III) oxide or charcoal, which promotes the otherwise slow symmetry-forbidden transition between ortho-hydrogen and the lower energy parahydrogen state) to ~20 K, a parahydrogen fraction of ~1 can be obtained (see Fig. 2b).

Parahydrogen itself is NMR silent since it has a total nuclear spin of 0; however, upon pairwise addition to magnetically-inequivalent sites on an unsaturated substrate molecule, the symmetry of the parahydrogen singlet state is broken and hyperpolarized ¹H MR signals can be observed. This hydrogenation reaction is typically performed in an organic solvent or the aqueous phase in the presence of a transition metal (typically Rh- or Ru-)based catalyst.⁴² The resulting ¹H nuclear spin state depends on the magnetic field at which parahydrogen addition is performed; at high field, e.g. within the MR system itself, the parahydrogen and synthesis allow dramatically enhanced nuclear alignment effect is observed,¹² whilst for hydrogenation at low field followed by adiabatic transport of the sample to the MR system for detection, the adiabatic longitudinal transport after dissociation engenders nuclear alignment effect is observed.⁴³ Several studies using PHIP of ¹H nuclei have been performed (e.g. to generate J-coupling derived contrast⁴⁴ and gas-phase imaging⁴⁵); however, due to the large background signal *in vivo* and lack of attainable pathophysiological functional information such as that pertaining to metabolism, heteronuclei such as ¹³C or ¹⁵N are of greater interest for biomedical applications. Polarization transfer from ¹H to heteronuclei is mediated by spin-spin couplings and can be driven by specialized RF pulse sequences^{46–48} or by subjecting the sample to a magnetic field cycle.^{49–51} The selection of polarization transfer method and its parameters depends on the configuration of the target molecular probe.^{52,53}

Regarding hardware, parahydrogen enrichment of ~50% can be achieved by simply flowing hydrogen gas through a cryogenic tube submersed in liquid nitrogen.⁵⁴ A high-throughput system to generate and store up to 50 bar of 98% parahydrogen has been developed for biomedical applications⁵⁵; once stored, parahydrogen enrichment can be maintained for months provided that paramagnetic molecular oxygen is not present.⁵⁶ Several automated PHIP polarizers for low-field hydrogenation and polarization transfer have been developed^{57–59} incorporating heated, high-pressure

spray reactors; however, promising results have also been obtained by simply shaking or bubbling of a parahydrogen-filled NMR tube followed by field cycling by hand (see e.g. Chukanov et al.⁶⁰). In addition, unlike d-DNP, it is possible to perform both the hydrogenation reaction and polarization transfer and generate heteronuclear hyperpolarization within the NMR magnet itself, minimizing the time for polarization decay.^{46,61}

d-DNP-polarized [1-¹³C]pyruvate: the pathway to clinical application

Abnormal metabolism is a hallmark of cancer, cardiovascular disease and other pathologies, and is intrinsically linked to inflammation and immune response.⁶² ¹⁸F fluorodeoxyglucose (FDG), a glucose analog, is routinely used for high-sensitivity and specificity clinical PET imaging of glucose metabolism⁶³ and is the recommended clinical indicator for head, neck, lung and pancreatic cancer.⁶⁴ However, since FDG-6-phosphate does not undergo further glycolysis, FDG-PET cannot probe metabolism beyond the first step of the glycolysis pathway. In this respect, d-DNP of [1-¹³C]pyruvate represents a significant development permitting unprecedented access to downstream metabolites to further aid understanding of cancer and disease mechanisms.

Whilst the first *in vivo* studies of a molecule polarization by d-DNP were performed with HP ¹³C-urea,⁶⁵ it was quickly realized that [1-¹³C]pyruvate, which plays a critical role in metabolism (see Fig. 3), is an ideal molecule for d-DNP since it is self-glassy and has long T₁ for ¹³C at the 1 and 2 positions (~40–60 s).⁶⁶ Golman et al.⁶⁷ demonstrated the first real-time metabolic imaging of metabolic production of [1-¹³C]lactate, [1-¹³C]alanine and [1-¹³C]bicarbonate from hyperpolarized [1-¹³C]pyruvate in healthy rats and pigs, and demonstrated differences in metabolite signal intensity in tumor tissues.⁶⁸ In cancer cells, glycolysis prevails over oxidative phosphorylation and the conversion of pyruvate to lactate via lactate dehydrogenase is up-regulated; this is known as the Warburg effect.⁶⁹ To date, increased HP [1-¹³C]pyruvate to [1-¹³C]lactate conversion has been used as the principal outcome of HP [1-¹³C]pyruvate MRI studies in several types of cancers.^{68,70–73} The high sensitivity of HP [1-¹³C]pyruvate MRI affords the possibility of non-invasive assessment of cancer treatment response, first demonstrated by Day et al.,⁷⁴ who showed a decrease in of HP ¹³C pyruvate–lactate flux after chemotherapy. The technique has since been applied in several studies of radiotherapy response^{75,76} and assessment of other treatments^{77,78} and reported to present a viable clinical alternative to FDG-PET for early tumor response in a preclinical study.⁷⁹

In a landmark paper, Nelson et al. reported the utilization of GE’s prototype sterile d-DNP system⁶ to perform the first *in-man* HP [1-¹³C]pyruvate MR spectroscopy and imaging feasibility study of patients with prostate cancer,⁴ demonstrating distinction of high- and low-grade tumors. This development has opened the door to realize real-time clinical metabolic imaging with HP [1-¹³C]pyruvate and the rapid

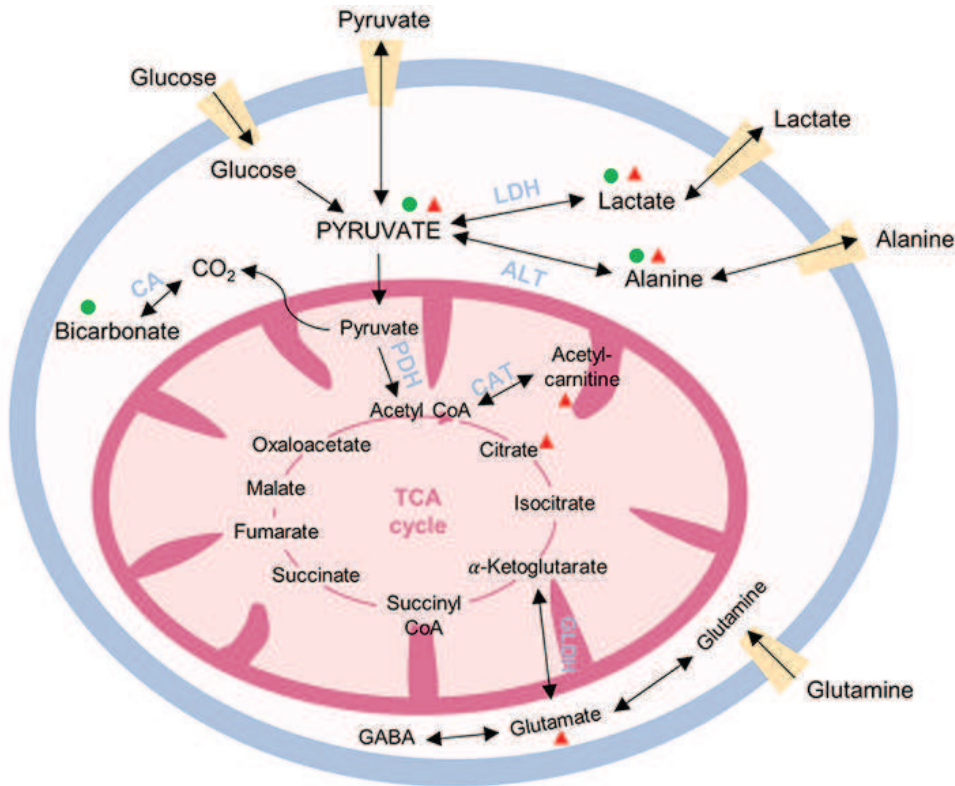


Fig. 3 Schematic of glycolysis, pyruvate metabolism to alanine and lactate, and the tricarboxylic acid (TCA) cycle within the mitochondria. Green circles: products of [1-¹³C]pyruvate; red triangles: products of [2-¹³C]pyruvate.

uptake of the technology is epitomized by the fact that more than 20 GE SpinLab polarizers have been installed worldwide, with close to half presently in use for human studies. First reports of the application of [1-¹³C]pyruvate to study metabolism in the healthy human heart⁸⁰ and brain⁸¹ have reported good tolerance of the procedure and contributed valuable reference data for interpretation of patient studies. In prostate cancer, HP [1-¹³C]pyruvate has been shown to detect early response to androgen deprivation therapy with a sensitivity exceeding that of T₂- and diffusion-weighted MRI.⁸² Preliminary reports in patients with liver metastases⁸³ and those with brain tumors^{84,85} demonstrate the wide range of potential targets of the technology and provide important pilot data for future trials. Several of these early clinical results are summarized in Fig. 4. Furthermore, at the 2019 International Society for Magnetic Resonance in Medicine (ISMRM) meeting, first HP [1-¹³C]pyruvate data in human patients with breast cancer, in which the relationship between intertumoral heterogeneity and gene expression analysis was investigated,⁸⁶ and preliminary *longitudinal* HP [1-¹³C]pyruvate data in glioma patients^{87,88} was reported, highlighting the advantages of the non-invasive nature of the technique for short- and long-term patient follow-up. Moreover, more than 30 clinical trials (sum of completed, ongoing and pending trials) are registered on the US and European clinical trials registries (summarized in Table 1) targeting a range of conditions, including prostate, brain, breast, ovarian, uterine, pancreatic and skin cancers, in addition to cardiovascular

indications and other brain pathologies. Comparison with FDG-PET to further comprehend the complementary information that can be obtained^{89,90} is a critical next step to aid interpretation of human HP [1-¹³C]pyruvate data and encourage further clinical dissemination.

As the number of clinical studies with [1-¹³C]pyruvate increases, there is a growing need for robust quantitation methods that can be applied universally for multi-site validation studies.⁹¹ Typically, HP [1-¹³C]pyruvate MR examinations include dynamic spectroscopy of the time-course of metabolic conversion of pyruvate, in addition to imaging. Semi-quantitative analysis of metabolic dynamics measured by MR spectroscopy can be performed using one of several models that have been developed to describe the rate of pyruvate–lactate conversion k_{PL} .⁹² For the most simple two-compartment model of pyruvate–lactate conversion, written in matrix form (see e.g. Harrison et al.⁹² and Harris et al.⁹³):

$$\frac{d}{dt} \begin{bmatrix} P_z \\ L_z \end{bmatrix} = \begin{bmatrix} -k_{PL} - \rho_P & k_{LP} \\ k_{PL} & -k_{LP} - \rho_L \end{bmatrix} \begin{bmatrix} P_z \\ L_z \end{bmatrix} \quad (3)$$

where P_z and L_z are the z-magnetization of pyruvate and lactate, respectively, k_{LP} is the (reverse) lactate–pyruvate conversion rate and $\rho_i = 1/T_{1,i} - \log(\cos(\alpha))/TR$ describes T₁ relaxation and RF-induced depolarization [cf. Equation (2)]. This equation can be analytically⁷⁴ or numerically solved and utilized to fit the magnetic resonance spectroscopy

(MRS) signal intensities of lactate and pyruvate (see for example the data in Fig. 4a) to yield k_{PL} as a metric of the Warburg effect. Model-free approaches such as the area under the signal-time curve and time-to-peak present simple, robust alternatives.⁹¹ CSI-based techniques yield individual images for each metabolic product, and ratio maps of lactate to pyruvate signal intensity are commonly used to provide some degree of quantitation in a regional manner.

d-DNP beyond [1-¹³C]pyruvate: other candidate molecular probes

The range of molecular imaging targets that can be polarized by d-DNP is vast and an exhaustive list⁹⁴ is beyond the scope of the present article. In the following, we introduce several of the most promising d-DNP-polarizable ¹³C molecular probes for biomedical applications (see Table 2 for a summary).

While the large majority of pre-clinical and clinical studies to date have exploited the sensitivity of HP [1-¹³C] pyruvate to the Warburg effect (i.e. pyruvate–lactate metabolism), the C₁ atom of the remaining pyruvate that enters into the mitochondria is oxidized to CO₂ and subsequently converted to bicarbonate, and thus cannot be used to probe tricarboxylic acid (TCA) cycle metabolism. However, the C₂ atom passes to acetyl-CoA and enters into the TCA cycle, exhibiting several metabolic fates (Figs. 3 and 5b). Schroeder et al.²³ first reported detection of downstream metabolites including [1-¹³C]acetylcarnitine, [1-¹³C]citrate, [5-¹³C]glutamate in perfused rat hearts after injection of HP [2-¹³C]pyruvate, with decreased citrate and glutamate production post-ischemia. In response to rapid pacing challenge, *in vivo* measurements of cardiac metabolism showed increased [5-¹³C]glutamate production,⁹⁵ and increased glutamate,

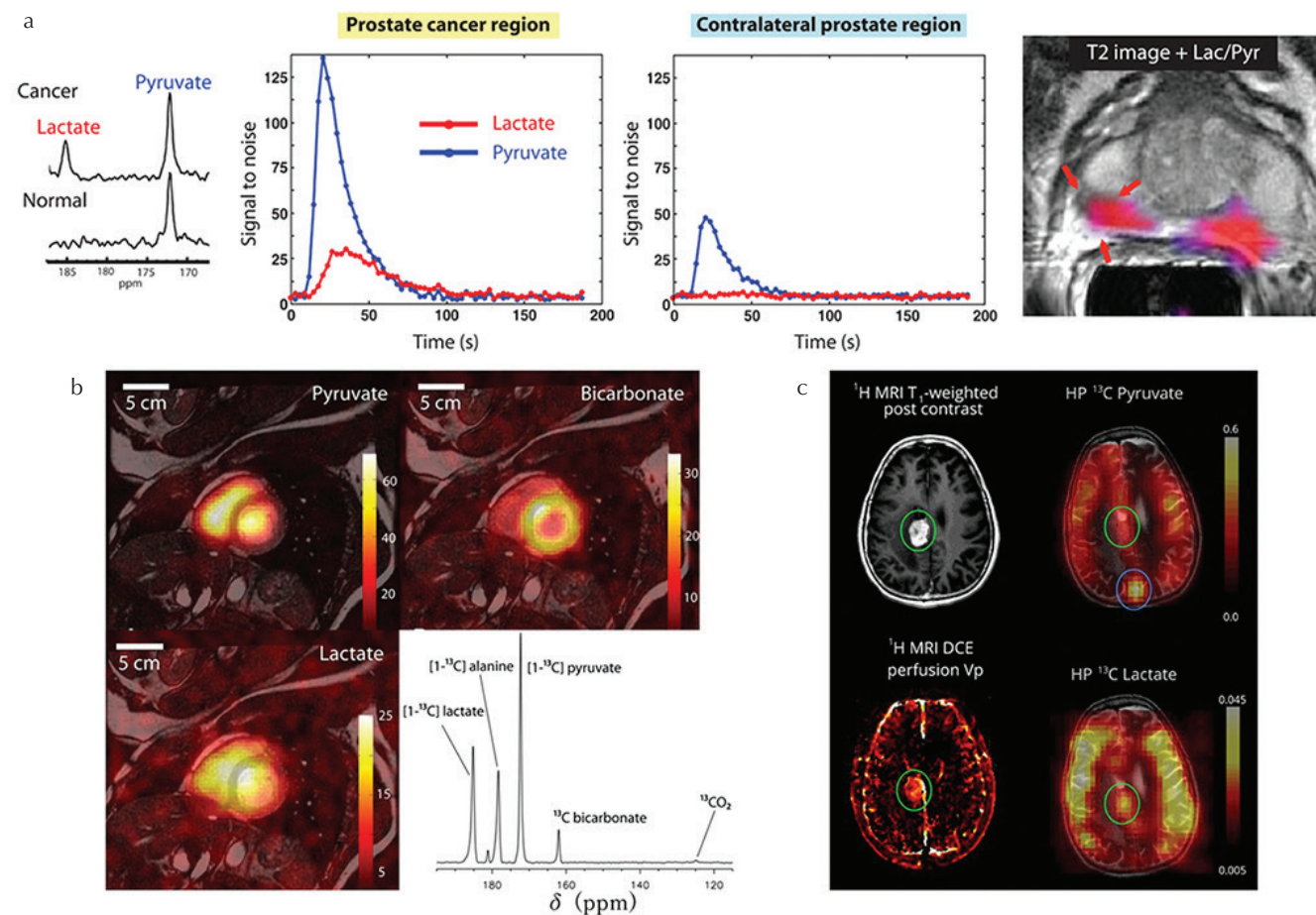


Fig. 4 Clinical examples of hyperpolarized [1-¹³C]pyruvate MRI. (a) Representative dynamic ¹³C MRS data of pyruvate and lactate signal in prostate cancer region and contralateral prostate region of a prostate cancer patient, and lactate/pyruvate signal ratio map overlaid on a T₂-weighted ¹H MR image (adapted from Figs. 2 and 4, respectively of Nelson et al.⁴ reprinted with permission from the American Association for the Advancement of Science (AAAS)). (b) HP [1-¹³C]pyruvate, lactate and bicarbonate MR images and a non-selective MR spectrum of the healthy human heart (adapted from Figs. 1 and 3, respectively of Cunningham et al.⁸⁰ reprinted with permission from Wolters Kluwer Health, Inc). (c) Comparison of HP [1-¹³C]pyruvate and lactate MR images to contrast-enhanced T₁-weighted MRI and perfusion plasma volume mapping in a patient with recurrent glioblastoma (adapted from Fig. 4 of Miloushev et al.⁸⁵ permission from the American Association for Cancer Research (AACR)).

Table 1 Summary of ongoing clinical trials pertaining to hyperpolarized ¹³C MRI (from clinicaltrials.gov, clinicaltrialsregister.eu and drks.de, accessed on 2019/06/12)

Primary condition (number of trials)	Participating center (country)	Enrollment [†]
Brain cancer ⁶	Sunnybrook Health Sciences Centre, Toronto (Canada)	121
	UT Southwestern Medical Center, Dallas (USA)	44
	M D Anderson Cancer Center, Dallas (USA)	13
	University of California San Francisco, San Francisco (USA)	80
	UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco (USA)	9
Uterine and ovarian cancer ²	Sunnybrook Health Sciences Centre, Toronto (Canada)	10
	Addenbrooke's Hospital, Cambridge (UK)	40
Breast cancer ²	UT Southwestern - Advanced Imaging Research Center, Dallas (USA)	110
	Sunnybrook Health Sciences Centre, Toronto (Canada)	13
Traumatic brain injury and CNS tumors ²	UT Southwestern Medical Center, Dallas (USA)	16
	Stanford University School of Medicine, Palo Alto (USA)	10
Other: Sarcoma ¹	Advanced Imaging Research Center, Dallas (USA)	20
Fatty liver ¹	UT Southwestern Medical Center, Dallas (USA)	16
Pancreatic cancer ¹	Aarhus University Hospital, Aarhus (Denmark)	15
Skin cancer ¹	Aarhus University Hospital, Aarhus (Denmark)	30
General cancer ¹	Memorial Sloan Kettering Cancer Center, New York (USA)	84
Prostate cancer ⁹	University of California San Francisco, San Francisco (USA)	261
	Sunnybrook Health Sciences Centre, Toronto (Canada)	40
	M D Anderson Cancer Center, Dallas (USA)	10
	University of California San Francisco, San Francisco (USA)	261
Cardiovascular disease ⁵	UT Southwestern Medical Center, Dallas (USA)	10
	Sunnybrook Health Sciences Centre, Toronto (Canada)	112
	University College London, London (UK)	25
	University Hospital Zurich, Zurich (Switzerland)	50
	Aarhus University Hospital, Aarhus (Denmark)	20

[†]Enrollment: approximate patient numbers scanned or anticipated (in cases of multiple studies at the same center, enrollment represents a summation of the enrollment for each individual study).

Table 2 Non-exhaustive list of ¹³C MR molecular probes polarizable by dynamic nuclear polarization (adapted with the publisher's permission from Table 1 of Hurd et al.¹⁶³) and their chemical shift (and literature reference)

HP ¹³ C probe (chemical shift)	Metabolic products (chemical shift)	Biomedical applications
[1- ¹³ C]Pyruvate (173 ppm) ¹⁶⁴	[1- ¹³ C]Lactate (185 ppm), [1- ¹³ C]alanine (178 ppm), [1- ¹³ C]bicarbonate (162 ppm), [1- ¹³ C]pyruvate hydrate (181 ppm) ¹⁶⁴	Warburg effect (cancer)
[2- ¹³ C]Pyruvate (208 ppm) ⁹⁶	[2- ¹³ C]Lactate (71 ppm), ⁹⁶ [2- ¹³ C]alanine (53 ppm), [1- ¹³ C]citrate (180–181 ppm), ¹⁶⁵ [5- ¹³ C]glutamate (184 ppm), [1- ¹³ C]acetylcarnitine (175 ppm), [3- ¹³ C]acetoacetate (177 ppm) ⁹⁶	Tricarboxylic acid (TCA) cycle metabolism
¹³ C-Urea (162.5 ppm) ¹⁰⁰	None (end product)	Perfusion
[1,4- ¹³ C ₂]Fumarate (175.4 ppm) ¹⁰³	[1- ¹³ C]Malate (181.8 ppm), [4- ¹³ C]Malate (180.6 ppm) ¹⁰³	Cellular necrosis
[1- ¹³ C] Dehydroascorbate (174.0 ppm) ¹⁰⁹	[1- ¹³ C]Ascorbic acid (vitamin C) (177.8 ppm) ¹⁰⁹	Redox status
¹³ C-Bicarbonate (161 ppm) ¹¹³	Carbon dioxide (125 ppm) ¹¹³	pH mapping
[1,5- ¹³ C ₂]Zymonic acid (ppm _{urea} + 10–15 ppm) ^{116*}	None	
[5- ¹³ C]Glutamine (178.5 ppm) ¹⁶⁶	[5- ¹³ C]Glutamate (181.5 ppm) ¹⁶⁶	Glutaminase metabolism,
[1- ¹³ C]α-ketoglutarate (172.6 ppm) ¹¹⁷	[1- ¹³ C]Glutamate (177.5 ppm) ¹¹⁷	TCA cycle metabolism
[1- ¹³ C]Acetate (182.5 ppm) ¹²⁰	[1- ¹³ C]Acetylcarnitine (202.1 ppm) ¹²⁰	Acetyl-CoA synthetase activity

*pH-dependent chemical shift.

acetoacetate and acetylcarnitine production was observed post-injection of an anti-cancer agent in rats.⁹⁶ The first clinical MR spectroscopy and imaging data of HP [2-¹³C]pyruvate in the healthy human brain was reported at the 2019 ISMRM meeting,⁹⁷ however, application of the probe remains challenging due to the relatively low concentration of downstream metabolites generated; in one study, none were detectable.⁶⁶

[1-¹³C]urea, the first hyperpolarized ¹³C molecular MR imaging agent demonstrated by the d-DNP method,¹¹ is metabolically inert and shows promise as a HP MRI agent for perfusion assessment.^{98,99} Furthermore, [1-¹³C]urea can be co-polarized with [1-¹³C]pyruvate for simultaneous assessment of metabolism and perfusion,¹⁰⁰ and co-labeling with ¹⁵N₂ exhibits prolonged ¹³C relaxation times and improved SNR¹⁰¹ facilitating for example the investigation of renal functional changes.¹⁰²

[1,4-¹³C₂]fumarate can be hyperpolarized by d-DNP and the rate of its conversion to malate, catalyzed by fumarase, is indicative of cellular necrosis.¹⁰³ HP [1,4-¹³C₂]fumarate exhibits high sensitivity to necrosis in myocardial infarction¹⁰⁴ and acute kidney injury¹⁰⁵ among other tissue pathologies, is complementary to [1-¹³C]pyruvate in the assessment of treatment response (Fig. 5a) in breast cancer¹⁰⁶ and efficient co-polarization schemes offer simultaneous probing of multiple metabolic pathways.¹⁰⁷

Hyperpolarization of the reduced and oxidized forms of vitamin C—namely [1-¹³C]dehydroascorbate and [1-¹³C]ascorbate, respectively—offers a novel means to probe intracellular redox status, a critical factor in normal and abnormal cellular function.^{108,109} High concentrations of [1-¹³C]ascorbate can be observed post-injection of [1-¹³C]dehydroascorbate, and reduced HP [1-¹³C]ascorbate signal has been utilized as an MR biomarker of renal oxidative stress.^{110,111}

Several HP ¹³C-based molecular probes have been proposed for measurement of pH,¹¹² a critical physiological factor. In particular, injection of hyperpolarized ¹³C-bicarbonate and monitoring of its conversion to ¹³CO₂ has been proposed to monitor pH¹¹³ and demonstrates sensitivity to abnormal pH in cancer¹¹³ and ischemic heart disease.¹¹⁴ An alternative method involves monitoring the HP ¹³CO₂ production from injected [1-¹³C]pyruvate.¹¹⁵ Recently, HP [1,5-¹³C₂]zymonic acid has been proposed for high-sensitivity *in vivo* pH mapping, exhibiting a pH-sensitive chemical shift and T₁ benefits over [1-¹³C]bicarbonate.¹¹⁶

To probe glutaminase and alanine transaminase metabolism, respectively, HP [5-¹³C]glutamine and [1-¹³C]glutamate have been investigated. Conversion of injected HP [1-¹³C]α-ketoglutarate to [1-¹³C]glutamate has been proposed as a potential biomarker of isocitrate dehydrogenase 1 gene mutations in glioma.¹¹⁷ Although the longitudinal relaxation of ¹³C nuclear spins in the glucose molecule is extremely short, perdeuteration has facilitated studies of glycolysis using HP [U-¹³C]glucose in cells¹¹⁸ and *in vivo*.¹¹⁹ The action of acetyl-CoA synthetase in generating acetyl-CoA—a

crucial molecule in fatty acid synthesis and TCA cycle metabolism—has been investigated with HP [1-¹³C]acetate in the heart^{120,121} and skeletal muscle.¹²²

PHIP: candidate ¹³C molecular and metabolic MRI probes

The choice of molecular probes for conventional hydrogenative PHIP is fundamentally limited by the requirement of an unsaturated precursor substrate (i.e. a molecule containing a double or triple bond to which parahydrogen is added to yield the hyperpolarized probe).¹²³ Nevertheless, a number of promising HP ¹³C probes for biomedical MR applications can be produced with a polarization level comparable to or approaching that of d-DNP. Some of these are highlighted in the following text and also in Table 3; for an exhaustive list, we refer the reader to Hövener et al.⁴¹

To date some of the most promising probes for metabolic MRI by PHIP are based on succinate and its derivatives (Fig. 6), the metabolic activity of which was introduced earlier. Hyperpolarized [1-¹³C]succinate can be generated by one of two PHIP strategies: two-step parahydrogen addition, first to [1-¹³C]acetylenedicarboxylate (ADC) to yield [1-¹³C]maleate, to which parahydrogen is added again to yield [1-¹³C]succinate¹²⁴; or by single-step parahydrogen addition to [1-¹³C]fumarate.^{125,126} The latter method offers a prolonged [1-¹³C]succinate polarization lifetime, particularly if deuterated fumarate is used, and also reduces the risk of undesired injection of ADC, which is mildly toxic, and also the intermediate (maleate).¹²⁵ Whilst initial *in vivo* experiments in the rat brain did not exhibit clear metabolic conversion of PHIP-polarized [1-¹³C]succinate,¹²⁴ the second hydrogenation approach enabled detection of downstream TCA cycle metabolites in a murine tumor model.¹²⁶ Furthermore, the diethyl ester of [1-¹³C]succinate, derived by parahydrogen addition of diethyl[1-¹³C]fumarate, appears to exhibit some TCA cycle metabolic sensitivity and was shown to distinguish murine tumor characteristics.^{126,127}

Hyperpolarized hydroxyethyl [1-¹³C]propionate, produced by parahydrogen addition of hydroxyethyl[1-¹³C]acrylate (HEA), presents a potential high-sensitivity PHIP contrast agent for angiography applications.^{50,128,129} In a recent study, the entire process of parahydrogen addition to HEA followed by polarization transfer, injection and *in vivo* MRI detection of HEP was realized within an MRI system, i.e. without the requirement of an external polarizer.¹³⁰ Since 2-hydroxyethyl[1-¹³C]propionate is easily polarized by PHIP and has strong, well-defined heteronuclear spin-spin couplings, it has also been utilized to validate several novel techniques for optimization of polarization transfer between parahydrogen and ¹³C.^{50,131,132}

Hyperpolarized tetrafluoropropyl[1-¹³C]propionate (TFPP) can be derived parahydrogen addition of the corresponding acrylate precursor and subsequent polarization transfer, and has been proposed as a “targeted” molecular agent for interrogating lipid-rich atherosclerotic plaques.¹³³ However, whilst HP ¹³C-HEP and ¹³C-succinate can be generated in the

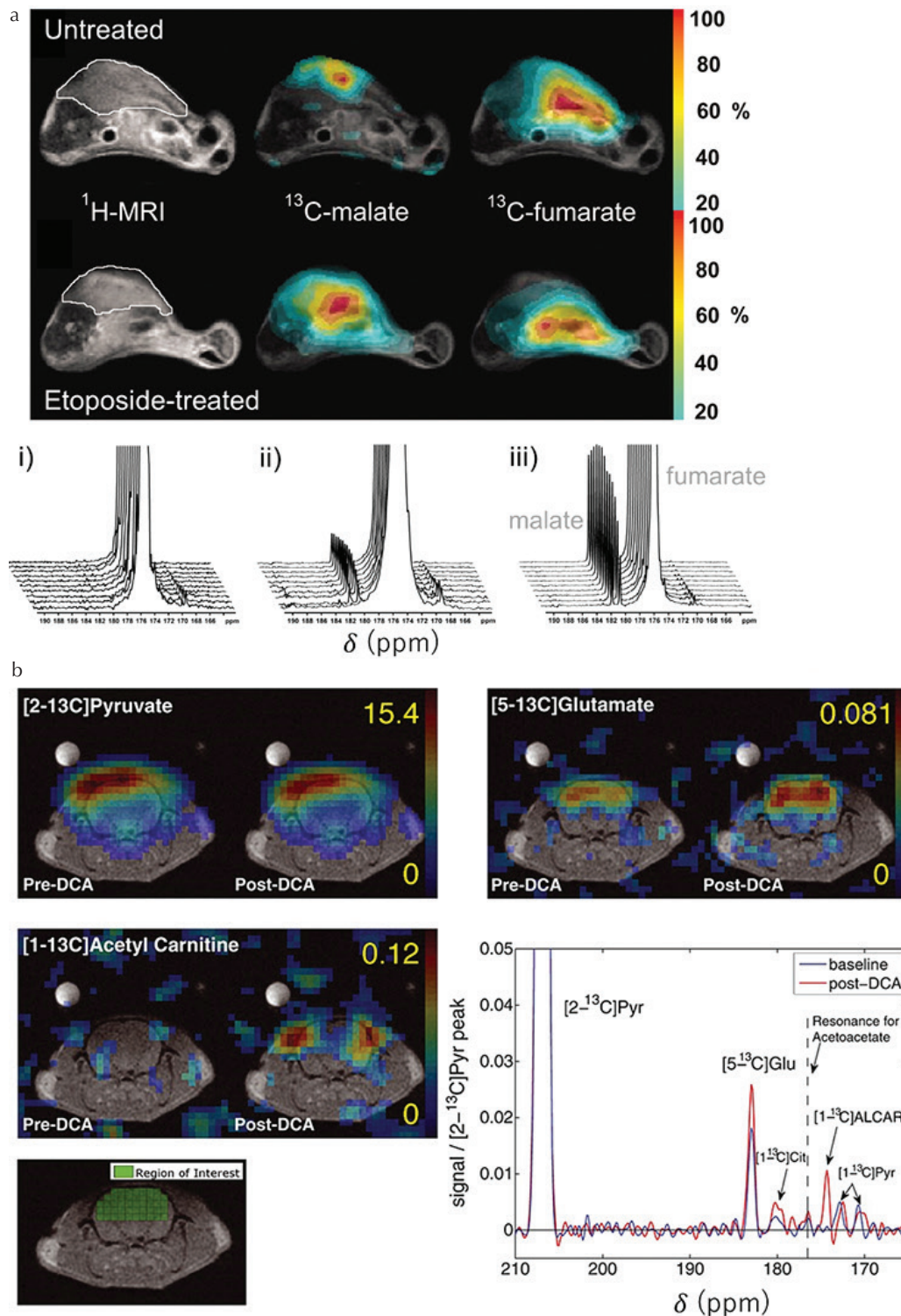


Fig. 5 Pre-clinical MRI examples of promising HP ^{13}C probes other than $[1-^{13}\text{C}]$ pyruvate. (a) HP ^{13}C chemical shift imaging (CSI) of cellular necrosis pre- and post-etoposide treatment (increased necrosis) in a murine tumor model after HP $[1,4-^{13}\text{C}_2]$ fumarate injection, and ^{13}C MR spectra obtained from murine lymphoma cells; (i) untreated, (ii) post-etoposide treatment, (iii) lysed cells, demonstrating a strong relationship between malate production and necrosis (adapted from Figs. 1 and 4 of Gallagher et al.¹⁰³ with the publisher's permission). (b) CSI-derived maps and accompanying spectra of metabolites derived from mitochondrial metabolism after injection of $[2-^{13}\text{C}]$ pyruvate into a healthy rat, exhibiting $[1-^{13}\text{C}]$ acetyl carnitine and tricarboxylic acid (TCA) cycle-derived $[5-^{13}\text{C}]$ glutamate resonances (adapted with the publisher's permission from Park et al.¹⁶⁷). Results obtained pre- and post-injection of dichloroacetate (DCA), a proposed anti-cancer drug used to influence acetyl-CoA production by modulating pyruvate dehydrogenase, are shown.

Table 3 Non-exhaustive list of ^{13}C MR molecular probes polarizable by parahydrogen-induced polarization (adapted with the publisher's permission from Table 1 of Hövener et al.⁴¹ and their chemical shift (and literature reference[†]))

HP ^{13}C precursor	Hydrogenation products	Biomedical applications
[1- ^{13}C]Acetyl dicarboxylic acid (151.6 ppm) ¹⁵⁵	[1- ^{13}C]Maleate (160 ppm) \rightarrow [1- ^{13}C]Succinate (175 ppm) ¹²⁴	Tricarboxylic acid (TCA) cycle metabolism
[1- ^{13}C]Fumarate (166.5 ppm) ¹⁵⁵	[1- ^{13}C]Succinate (175 ppm) ¹²⁴	
Diethyl[1- ^{13}C]fumarate (167.4 ppm) ¹²⁷	Diethyl[1- ^{13}C]succinate (175.8 ppm) ¹²⁷	TCA cycle metabolism
^{13}C -Hydroxyethyl-acrylate	^{13}C -Hydroxyethylpropionate (~180 ppm) ⁵⁰	Angiography
Tetrafluoropropyl[1- ^{13}C]acrylate	Tetrafluoropropyl[1- ^{13}C]propionate (174 and 177 ppm) ¹³³	Atheromatous plaques
[1- ^{13}C]Phosphoenol-pyruvate (171.9 ppm) ¹³⁵	[1- ^{13}C]Phospholactate \rightarrow [1- ^{13}C]Lactate (182.1 ppm) ¹³⁵	Gluconeogenesis, lactate dehydrogenase metabolism
Propargyl[1- ^{13}C]pyruvate (160 ppm) ⁶⁰	Allyl[1- ^{13}C]pyruvate (160.5 ppm) ⁶⁰ \rightarrow [1- ^{13}C]pyruvate (173 ppm) after hydrolysis	Warburg effect (cancer)
Vinyl[1- ^{13}C]acetate (168 ppm) ⁶⁰	Ethyl[1- ^{13}C]acetate (174 ppm) ¹⁴⁷ \rightarrow [1- ^{13}C]acetate (182.5 ppm) after hydrolysis	Acetyl-CoA synthetase activity

[†]Chemical shift values only quoted for the particular solvent in the literature reference cited.

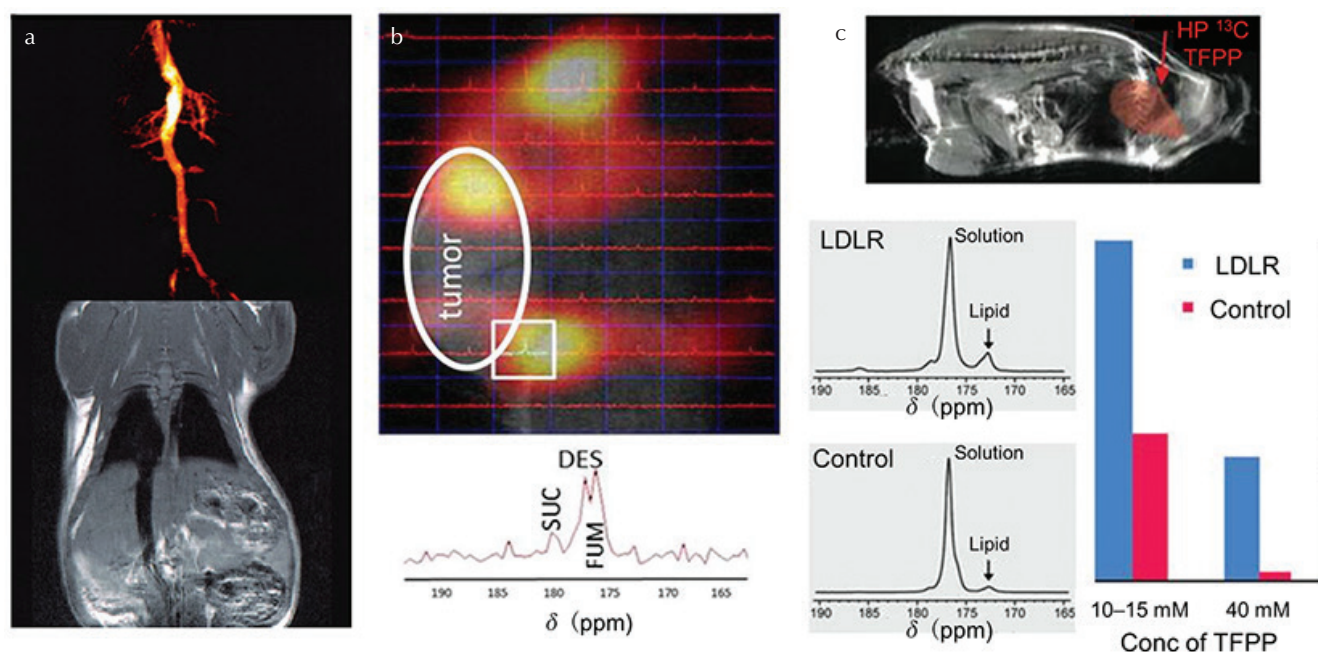


Fig. 6 *In vivo* magnetic resonance imaging (MRI) application of several hyperpolarized ^{13}C probes generated by parahydrogen-induced polarization (PHIP). (a) MRI angiogram of HP ^{13}C -labeled malate dimethyl ester with corresponding ^1H spin echo reference image of a healthy rat (adapted with permission from Golman et al.⁴⁹). (b) Chemical shift imaging (CSI) of HP diethyl [1- ^{13}C]succinate in a murine model of renal cell carcinoma (reproduced from Zacharias et al.¹²⁶ under the Creative Commons Attribution License). The ^{13}C spectrum corresponding to the pixel indicated by the white square shows tricarboxylic acid (TCA) cycle metabolism of diethyl succinate (DES) to succinate (SUC) and fumarate (FUM). (c) Representative HP tetrafluoropropyl [1- ^{13}C]propionate (TFPP) fast imaging with steady-state precession (FISP) image overlaid on a ^1H RARE image, and HP ^{13}C -TFPP spectra obtained from low density lipoprotein receptor (LDLR) deficient mice compared with control mice, demonstrating excess lipid in LDLR mice (reproduced from Bhattacharya et al.¹³³ with the publisher's permission).

pure aqueous phase using a water-soluble catalyst, TFPP requires a high dose of ethanol as a co-solvent, limiting potential *in vivo* applications.¹³³

Since [1- ^{13}C]ethyl pyruvate ester has been shown to be polarizable by d-DNP and shows some promise in comparison to [1- ^{13}C]pyruvate for functional brain imaging

applications,¹³⁴ the hydrogenation precursor [1- ^{13}C]vinyl pyruvate is an interesting potential target for PHIP, however an efficient synthesis route remains elusive.⁶⁰

Shchepin et al.¹³⁵ have proposed [1- ^{13}C]phospholactate, the hydrogenation product of [1- ^{13}C]phosphoenolpyruvate, as a possible route to HP [1- ^{13}C]lactate *in vivo*, which

is subsequently taken up by tumors and several critical organs.^{59,136} The hydrogenation reaction can relatively easily be performed in water,¹³⁷ which holds promise for future biomedical studies.

Ester derivatives of ^{13}C -glucose have been demonstrated to be polarizable by PHIP;¹³⁸ however, the short polarization lifetime (\sim s) must be overcome (e.g. by deuteration) to facilitate the realization of *in vivo* glycolysis measurement by PHIP of glucose derivatives and the possibility of corroboration against FDG-PET.

Alteration of choline metabolism is a hallmark of tumor progression, and several groups have investigated choline precursors as potential molecular probes for PHIP.^{139,140} Rather than ^{13}C , ^{15}N -labeling can be used; although ^{15}N possesses an intrinsically low gyromagnetic ratio and hence sensitivity compared with ^{13}C , extremely long relaxation times can be realized, enabling metabolism dynamics to be followed over the course of several minutes. In particular, the recent demonstration of 12% ^{15}N polarization with a lifetime of over 20 min on a choline derivative is of interest for *in vivo* cancer metabolism applications.¹⁴¹

Side-arm hydrogenation (PHIP-SAH): a route to HP $[1-^{13}\text{C}]$ pyruvate

The majority of the above-mentioned probes offer only limited or no metabolic information of sufficient sensitivity compared with $[1-^{13}\text{C}]$ pyruvate produced by d-DNP; however, the lack

of a suitable hydrogenation precursor of pyruvate, lactate or other metabolically-linked molecules has led Reineri et al.¹⁴² to develop the method of side-arm hydrogenation PHIP (PHIP-SAH). In PHIP-SAH, parahydrogen is added to an unsaturated ester of the molecule of choice in the organic phase, where the hydrogenation reaction is most efficient, then polarization is transferred from ^1H to the $[1-^{13}\text{C}]$ atom of the carboxylic acid of interest, and finally the ester “side-arm” is hydrolytically cleaved to yield the HP carboxylic acid of interest along with ester alcohol in the aqueous phase. Hyperpolarized $[1-^{13}\text{C}]$ pyruvate, $[1-^{13}\text{C}]$ acetate¹⁴² and $[1-^{13}\text{C}]$ lactate¹⁴³ have been demonstrated using this approach.

Following optimization of the initial experimental procedure with a view to *in vivo* application,¹⁴⁴ a ^{13}C polarization of \sim 5% on $[1-^{13}\text{C}]$ pyruvate at the time of experiment was obtained, enabling realization of the first *in vivo* metabolic MR spectroscopy and imaging in a mouse model of dilated cardiomyopathy,¹⁴⁵ the results of which are highlighted in Fig. 7. Whilst the sensitivity remains relatively low compared with that produced by d-DNP, a recent comparison of the polarization efficiency of several pyruvate and acetate precursors has provided insights into the best substrate of choice for future *in vivo* metabolic MRI applications.¹⁴⁶ In particular, hydrogenation products ethyl acetate and allyl pyruvate (hydrogenation products of vinyl acetate and propargyl pyruvate, respectively) were found to yield the highest ^{13}C polarization.¹⁴⁶ Furthermore, when a deuterated

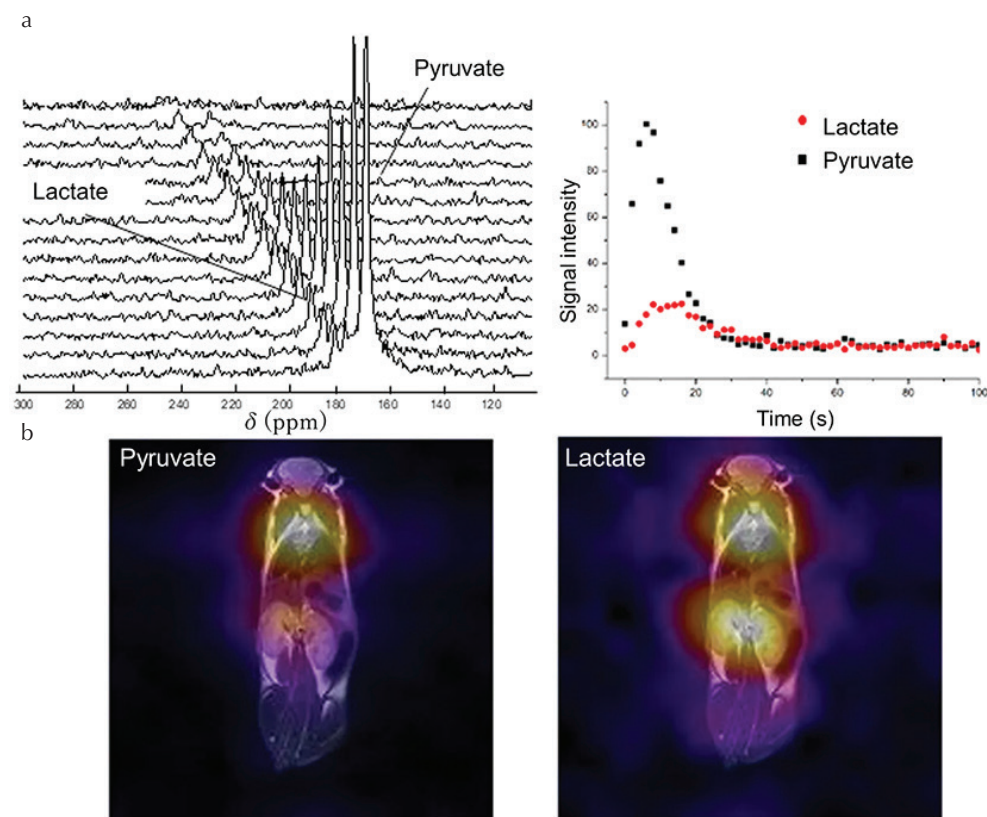


Fig. 7 (a) Slice-selective dynamic ^{13}C MRS of a healthy wild-type mouse after injection of HP $[1-^{13}\text{C}]$ pyruvate produced by parahydrogen-induced polarization (PHIP)-side-arm hydrogenation (SAH), and (b) corresponding whole-body ^{13}C chemical shift imaging (CSI) of $[1-^{13}\text{C}]$ pyruvate and $[1-^{13}\text{C}]$ lactate (reproduced from Figs. 2 and 3 of Cavallari et al.¹⁴⁵ under the Creative Commons CCBY License).

precursor is combined with optimized polarization transfer techniques, ^{13}C polarization of more than 50% on acetate has been realized using the vinyl ester precursor,¹⁴⁷ which may permit *in vivo* investigations of acetyl-CoA synthetase activity in the near future by PHIP.

Future Perspectives

Ongoing and future clinical trials of $[1-^{13}\text{C}]$ pyruvate MRI serve a critical role in evaluating the clinical viability of the technique for and beyond oncological studies of metabolism, and also in assessing the reproducibility and robustness of hyperpolarized MR acquisition methods and analysis procedures in order to provide guidelines to standardize workflow for future multi-site validation studies.⁵ In particular, robust clinical comparison studies of HP $[1-^{13}\text{C}]$ pyruvate MRI and ^{18}F -FDG-PET in several oncological pathologies are required to further understanding of the relationship between the pathophysiological information gleaned from each technique and further accelerate clinical translation.^{89,90} Clinical trials of d-DNP probes such as $[1-^{13}\text{C}]$ fumarate, $[1-^{13}\text{C}]$ bicarbonate and others are either pending or expected in the near future, and co-polarization techniques are likely to yield unprecedented access to multiple aspects of metabolic function with a single hyperpolarized dose.^{107,148} d-DNP probe development has not ceased with the advent of clinical application of $[1-^{13}\text{C}]$ pyruvate, with several novel probes reported in the last few years.^{149,151} In parallel to clinical studies, the fundamental science of d-DNP remains a field of active development.¹⁵²

Whilst biomedical applications of PHIP are relatively few in number to date when compared with those of d-DNP, novel approaches such as PHIP-SAH offer an expanded palette of polarizable molecular targets and a low-cost means of generating HP $[1-^{13}\text{C}]$ pyruvate for preclinical and with further refinement, eventually clinical applications.^{142,145} In addition, the development of increasingly efficient and versatile hydrogenation catalysts is a thriving research field (see e.g. Glöggler et al.,¹⁵³ Leutzsch et al.¹⁵⁴). In particular, rhodium-based catalysts commonly used for efficient hydrogenation predominantly yield *cis*-selective products, but a novel *trans*-selective ruthenium-based catalyst has recently been shown to demonstrate hyperpolarized $[1-^{13}\text{C}]$ fumarate by parahydrogen addition to acetylene $[1-^{13}\text{C}]$ dicarboxylate for the first time.¹⁵⁵ With appropriate filtering of the catalyst¹⁵⁶ and other unwanted co-solvents or hydrolysis side products (in the case of PHIP-SAH), the purity of injected doses can be improved to appropriately high levels with a view to clinical application in the foreseeable future.

It is not only the ^{13}C nucleus that shows promise for biomedical hyperpolarized MRI applications; as previously noted, the ^{15}N nucleus has a relatively low MR sensitivity, but exhibits extremely long polarization lifetimes and metabolic probes can be prepared in an environment suitable for biological application, analogous to ^{13}C .^{157,158} In addition, ^{19}F , which has a gyromagnetic ratio and therefore a baseline sensitivity

similar to that of the proton, may find biomedical application in targeted MRI of hyperpolarized ^{19}F -labelled drugs, though limited progress in this direction has been made to date.¹⁵⁹ Furthermore, while all the above noted applications pertain to liquid-phase molecular probes, parahydrogen can be used in combination with a solid-phase catalyst to generate ^1H -hyperpolarized propane (from propylene) in the gaseous phase.^{160,161} which shows some promise as a relatively cheap alternative to hyperpolarized noble gases for biomedical lung imaging, though the high ^1H background signal may be problematic and no *in vivo* experiments have been attempted to date.

Finally, the SABRE parahydrogen method, wherein polarization transfer occurs by reversible exchange and the target molecule remains chemically unaltered upon interaction with parahydrogen, has the potential yield heteronuclear (^{13}C , ^{15}N) hyperpolarization on a broader range of molecular imaging probes than conventional PHIP and may lead to several unprecedented avenues of biomedical application.¹⁶² Although to date no *in vivo* experiments have been performed with SABRE-polarized probes, the recent demonstration of both hyperpolarized $[1-^{13}\text{C}]$ and $[2-^{13}\text{C}]$ pyruvate,¹⁴ although at relatively low polarizations, represents a significant step toward biomedical application.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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