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The Use of Hyperpolarized Carbon-13 Magnetic Resonance for Molecular Imaging

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Abstract

Until recently, molecular imaging using magnetic resonance (MR) has been limited by the modality's low sensitivity, especially with non-proton nuclei. The advent of hyperpolarized (HP) MR overcomes this limitation by substantially enhancing the signal of certain biologically important probes through a process known as external nuclear polarization, enabling real-time assessment of tissue function and metabolism. The metabolic information obtained by HP MR imaging holds significant promise in the clinic, where it could play a critical role in disease diagnosis and therapeutic monitoring. This review will provide a comprehensive overview of the developments made in the field of hyperpolarized MR, including advancements in polarization techniques and delivery, probe development, pulse sequence optimization, characterization of healthy and diseased tissues, and the steps made towards clinical translation.

Keywords

Hyperpolarization; DNP; PHIP; CSI; MRSI; Pyruvate; Lactate; Metabolic Imaging; Molecular Imaging; Cellular Imaging

1. Introduction

Molecular imaging refers to a gamut of imaging modalities that—as succinctly defined by the Radiological Society of North America (RSNA)—“directly or indirectly monitor or record the spatiotemporal distribution of molecular or cellular processes for biochemical, biologic, diagnostic, or therapeutic applications” [1]. Numerous modalities fit this description, most prominently positron emission tomography (PET) and single photon emission computed tomography (SPECT), both of which are commonly used in the clinic

for diagnosing several disorders and monitoring therapeutic response [2]. More recently, fluorescent and bioluminescence optical imaging have allowed high-sensitivity molecular imaging in pre-clinical research, while computed tomography (CT)—traditionally considered a structural imaging tool—is now being used as a molecular imaging modality with the advent of cell- and tissue-specific nanoparticles and as a combined modality with PET or SPECT [3,4].

Clinical use of magnetic resonance imaging (MRI) has largely been limited to structural assessment. However, proton (^1H), sodium (^{23}Na), phosphorus (^{31}P), and carbon (^{13}C) MRI/nuclear magnetic resonance (NMR) are extensively used as molecular imaging tools in pre-clinical studies in animals and cells. In these studies, the modality's foremost limitation—its low sensitivity—can be overcome by the use of longer scan times and signal averaging [5–7]. As such, molecular MRI/NMR using conventional thermally polarized nuclei is limited to probing quasi-steady-state metabolism. The advent of hyperpolarized (HP) MRI/NMR overcomes this limitation by substantially enhancing the signal of certain biologically important probes through a process known as external nuclear polarization, enabling real-time molecular imaging with unprecedented temporal resolution. The information obtained by HP MR has significant promise in the clinic, where imaging-derived metabolomics could play a critical role in disease diagnosis and therapeutic monitoring.

Hyperpolarized MR is a burgeoning field in which investigators continue to make rapid advancements in an array of areas, which include polarization techniques and delivery, probe development, pulse sequence optimization, characterization of healthy and diseased tissues, and clinical translation. In light of the rapidly changing and multifarious nature of this technology, this review aims to provide a comprehensive overview of the current state of molecular imaging using HP MR, the developments that have led to this point, and the central challenges that hyperpolarized technology faces as it moves towards making a clinical impact.

2. Molecular Imaging using Non-MR Modalities

Several well-established molecular imaging techniques are actively used in basic and clinical research. This section describes applications of each technology, their advantages and limitations, and how they differ from HP MRI/NMR.

2.1. PET and SPECT

Positron emission tomography (PET) and single photon emission computed tomography (SPECT) use radioactively labeled metabolic tracers (e.g., ^{18}F -FDG,) to probe the cellular uptake of their analogous biologically relevant molecules (e.g., glucose). Both techniques are widely used in clinical [2,8,9] and pre-clinical [10–13] scenarios for a broad range of applications including, but not limited to, the diagnosis, staging, and monitoring of a variety of cancers [14–18], drug development—e.g., probing the bio-distribution of new pharmaceuticals [19]— and neuroimaging [20].

Although PET and SPECT provide high sensitivity for detection, they suffer from poor spatial resolution [4,21]. As a result CT or MRI are used for accurate reconstruction and

anatomical localization of tracers [22]. Another major shortcoming of both modalities is that they are limited to revealing abnormalities in the uptake or retention of the tracers; they are unable to reveal changes in downstream metabolites that may be crucial to the diagnosis and staging of diseases.

On the other hand, hyperpolarized MRI—which probes analogous pathways to PET—highlights alterations in downstream metabolites using their unique ^{13}C NMR resonance frequencies, thus providing non-invasive interrogation of the size of metabolite pools and the flux through their corresponding pathways [23]. However, unlike PET and SPECT, absolute quantification of HP MR substrates can be challenging due to variations in polarization level and the use of super physiological concentrations of administered agents [24]. Furthermore, despite the large signal gains achieved using hyperpolarization, the near-unity photon detection efficiency of radionuclide agents is not achievable. Nevertheless, hyperpolarized probes can provide complementary imaging information that address the same diagnostic imaging goals as PET and SPECT.

2.2. Fluorescence and Bioluminescence Tomography

Optical tomography is widely used for the preclinical study of biology and pathology. The major optical techniques, fluorescence and bioluminescence tomography, are based on different underlying mechanisms, but have similar applications and limitations. Fluorescence tomography uses injected or genetically inserted dyes and proteins which fluoresce at different wavelengths than normal tissue to tag specific cells or molecules and track their distribution, while bioluminescence tomography utilizes the intrinsic light emission of select molecules to similar effect [4,25]. In the latter case, light is produced when the light emitting pigment luciferin is catalyzed by the enzyme luciferase, which is produced by genetically modified cells that synthesize the enzyme.

Optical imaging benefits from minimal background tissue radiation and high photon detection efficiency, and therefore generally has an excellent signal-to-noise (SNR). Both modalities offer spatial resolution of approximately 2–3mm. However, the need for genetic modification of the cells to allow targeted molecular imaging limits their use within human patients; this is particularly an issue with bioluminescence imaging. Additionally, optical techniques suffer from low tissue penetrance and the related need for a high concentration of imaging agent to successfully perform studies of deeper tissue. There has recently been an interest in using fluorescent probes in applications that circumvent this limitation, such as for more accurate identification of malignancies during surgical resection [26]. While PET and hyperpolarized MRI interrogate analogous pathways, those targeted by optical imaging are typically very different, and as such the modality can provide distinct and perhaps complementary molecular information to the former two modalities.

2.3. Computed Tomography

Computed tomography (CT) has not traditionally been considered as a molecular imaging technique. However, recent developments in nanoparticle-bound contrast agents (CAs) have allowed for selective imaging of specific cell tissues and molecules using CT. Iodinated nanoparticles, for example, can change the biodistribution of the iodine upon injection

depending on the characteristics of the nanoparticle. This method has shown promise both in improving upon traditional CAs in blood pool imaging and in specifically labeling certain cell types in various applications including studying angiogenesis in tumor growth and response to therapy [27–31]. Iodinated nanoparticles have also been labeled with antibodies to increase their molecular specificity to probe expression of particular proteins [32]. Gold nanoparticles have also become popular due to their easy synthesis and low toxicity [33]. These particles have also shown promise in vascular imaging, tumor imaging, and bone tissue damage imaging [3,34–37].

Nanoparticle based CAs are an area of interest for industry due to the wide availability of clinical CT machines, and there are currently several clinical trials examining the safety of gold nanoparticle based contrast agents [38]. Although the use of iodine and gold nanoparticles is promising in molecular imaging, their primary role seems to be to provide better structural contrast and anatomic localization by targeting specific proteins or cell markers, as opposed to deriving metabolic or functional information. Furthermore, ionizing radiation remains a fundamental limitation of this technology when used for clinical studies.

2.4. Photoacoustic Tomography

Photoacoustic tomography images differences in tissue optical absorbance via the delivery of laser pulses. The energy from the laser absorbed by the tissue heats the tissue causing thermoelastic expansion of tissues resulting in the emission of ultrasonic waves that can be detected using modified ultrasound equipment, and converted into an image. Current techniques enable a depth of imaging ranging from ~6 mm to ~5 cm, and resolution on the 10 μ m to mm scale depending on the marker used [4]. Photoacoustic contrast agents such as methylene blue, single-walled carbon nanotubes (SWNTs) and gold nanoparticles can be tagged to specific molecules, allowing for molecular-level imaging. While there has been some recent interest in clinical applications of photoacoustic tomography in conjunction with ultrasound, the use of molecular contrast agents for photoacoustic tomography has largely been limited to preclinical studies, for example, 50-nm gold nanocages have been used to image sentinel lymph nodes in one study [39], and 50–300-nm carbon nanotubes have been used to detect prostate cancer in rats [40].

Photoacoustic imaging provides several benefits over fluorescence and bioluminescence imaging including deeper tissue penetration, lack of need for imaging agents, and higher temporal resolution. However, the technology suffers from an inability to penetrate bone or air filled structures, and a lower penetration depth compared to MRI, PET or CT.

3. Hyperpolarized MRI

Hyperpolarization refers to a process in which certain nuclei are aligned to a degree many orders of magnitude greater than is normally achievable under *in vivo* conditions. This leads to a magnetic resonance signal that greatly exceeds that which is otherwise available and makes qualitatively different applications possible, including imaging of gases and low-concentration biological molecules. If the non-equilibrium hyperpolarized state is to be useful for medical imaging, the alignment must persist long enough for the substance to be administered, arrive at the target organ or system, and perhaps be taken into cells and/or

metabolized. Typically, the persistent state can be achieved only if several conditions are met. First, the nuclei must be what is known as “spin-1/2”. Spin-1/2 nuclei have two distinct quantum states of orientation with respect to a magnetic field; all other classes of nuclei either have no magnetic moment (and are therefore invisible in MRI) or are susceptible to rapid reorientation by the large electric field gradients present in molecules and during atomic or molecular collisions. Second, the nuclei must be relatively segregated from all other magnetic moments, including unpaired electrons or magnetic nuclei on the same molecule. Third, any residual magnetic interactions with the nucleus to be hyperpolarized must be limited in duration or frequently randomized in direction, again in order to prevent loss of alignment in an extended nuclear precession around the depolarizing field.

As a practical matter, this limits hyperpolarization with biological applications to a few nuclear species that are found in *in vivo* (^{13}C , ^{15}N , ^{31}P and potentially ^1H), those found in some molecules that are not acutely toxic (^{19}F , ^{29}Si) and the two biologically inert gases ^3He and ^{129}Xe . Of these, the gases have been used primarily for imaging of lung function, although molecular biosensors and imaging agents based on ^{129}Xe have been proposed. Agents based on the other nuclei (primarily ^{13}C) have been studied more extensively in molecular imaging applications.

Two straightforward methods have been developed for transferring polarization from light to atomic nuclei, and have been used extensively for atomic gases [41,42]. Unfortunately, these techniques are not applicable to molecules [43]. This is primarily because the strong coupling between coherent rotational motion and nuclear spins causes rapid loss of spin alignment, but practical issues of chemical reactivity and short-lived electronic excited states are also difficult to overcome. There are two alternative approaches, however, that can lead to polarization of order unity of selected nuclei in biologically active compounds. These methods are dynamic nuclear polarization (DNP), and parahydrogen-induced polarization (PHIP).

3.1. Dynamic Nuclear Polarization (DNP)

DNP refers to a process in which the alignment of an unpaired electron is achieved at thermal equilibrium, and this alignment is transferred to a nearby nucleus via a process analogous to the Overhauser effect [44,45]. In order to prepare a sample suitable for DNP, the molecule of interest is mixed with a small amount (1 part in 100 or less) of stable free radical that supplies the unpaired electron spin. This mixture is then placed in conditions of reduced temperature and elevated magnetic field sufficient to achieve near-complete electron alignment; as a rule of thumb, the magnetic field (in Tesla) divided by the sample temperature (in Kelvin) must be approximately 1 to achieve full alignment. As an intimate mixture of radical and target molecule is necessary for spin order transfer, and favorable DNP conditions generally imply a frozen sample, additional components may be required to assure a homogeneous (glassed) mixture [46].

The transfer of polarization from electron to nucleus is accomplished by means of a microwave driving field tuned near the resonance frequency of the free electron in the chosen magnetic field. The mechanism of polarization transfer is complex and may differ between samples; however, the electron-nuclear interaction is typically described as one of

three processes: the solid effect, the cross effect, and thermal mixing. The solid effect refers to a mechanism in which two closely-spaced magnetic dipoles, one electron and one nucleus, simultaneously invert orientations with respect to the field. Because the vast majority of electrons are aligned, this results in the net polarization of the nuclear ensemble. This process is greatly suppressed by the large energy difference between electronic and nuclear Zeeman transitions, and is only relevant because of the very large number of coupled electron and nuclear pairs available in the sample. The cross effect refers to a related process in which one nucleus and two electrons, differing in frequency by approximately the nuclear Zeeman frequency, are excited simultaneously. Because this process is nearly energy-conserving, it is resonant over a duration consistent with typical solid state electron-nuclear couplings and is therefore quite rapid. However, the number of coupled three-spin systems with the appropriate resonance frequencies is relatively small; the relative contribution of solid effect and cross effect polarization is therefore dependent on the details of the sample's frozen state and external conditions such as temperature and magnetic field [47,48].

Under hyperpolarization conditions useful for molecular imaging studies, the distinction between the above two processes (and those involving more electrons) is obscured by the strong couplings between closely-spaced radical molecules. The polarization process is therefore best described by thermodynamic state transitions in the band-like electron system and couplings to the nuclear system. Incorporating aspects of the other two processes, this theory is termed thermal mixing. Although lacking an intuitive, conceptual explanation, the theory is well-established and successful, and is described in detail in the literature [47,49,50].

3.1.1. Practical Aspects of DNP for Biomolecules—In choosing appropriate conditions for efficient DNP, it is important to consider the magnetic properties of the molecule to be polarized. Small biological molecules typically consist of carbon, oxygen and hydrogen, often contain nitrogen, and sometimes contain other elements (e.g., phosphorus or iron). As a general rule, a nucleus that is suitable for hyperpolarization is spin- $\frac{1}{2}$ (to eliminate rapid loss of alignment due to electric field gradients) [51] and is distant from other magnetic nuclei. The latter consideration is necessary to avoid loss of alignment due to strong magnetic interactions in solution, and as it is usually impossible to eliminate all intramolecular magnetism, small molecules (in which interactions change direction frequently due to molecular reorientation) are more successful than large ones. Taken together, these characteristics of polarizable nuclei have suggested use of the spin- $\frac{1}{2}$ isotope ^{13}C , as it can be incorporated into nearly all biomolecules in the magnetically isolated carbonyl, quaternary and carboxylic acid forms. Small carboxylic acids in particular are near-ubiquitous as TCA cycle intermediates, amino acids and in many other biologically central functions. For this reason, nearly all of the *in vitro* and *in vivo* work described in this review makes use of this class of molecules.

Suitability of a molecule for hyperpolarization by DNP is also determined by the physical properties of the bulk or dissolved compound. Because the process takes place at cryogenic temperatures, all molecules of interest are in the solid phase; however, the intimate radical/biomolecule mixture necessary is best achieved through dissolution in the liquid. Substances that are gases at room temperature must be cooled or pressurized before mixture, and those

that are solids must be co-dissolved with the radical in a suitable solvent. Furthermore, the tendency of many solutions to segregate upon freezing must be avoided, typically by transitioning past the freezing temperature very quickly, and in many cases including a 'glassing agent' in solutions that would otherwise crystalize.

Many molecules of interest, particularly those with hydrophobic regions, are sufficiently insoluble in water that the sample to be hyperpolarized must also include additional ingredients to facilitate increased concentration. Thus, although some general strategies are useful in classes of similar molecules, each sample preparation is somewhat individual and is optimized experimentally. A detailed discussion of this process is beyond the scope of this article, but it is described in detail in an excellent review [52]. It is worth noting that one compound, pyruvic acid, is highly favorable from all of the above points of view; it is a small (a three-carbon chain, molecular weight 88 Da) carboxylic acid, when ^{13}C -labeled in the 1 position the nucleus is remarkably isolated (the nearest magnetic nucleus is three bonds away) such that relaxation of the hyperpolarized state is slow [53], the neat acid is liquid at room temperature and glasses without additional ingredients when frozen. Furthermore, pyruvate is metabolized rapidly by most cells, and the metabolic fate is indicative of cellular redox state and other biologically important factors. As is apparent in subsequent sections of this article, this fortuitous set of circumstances has led to pyruvate's central role in the development of hyperpolarized agents.

The final aspect of DNP hyperpolarization under the user's control is the choice of radical species. In the development of the technology, several types of radical were explored, with considerable attention paid to nitroxide moieties and those derived from the triphenylmethyl (trityl) radical. In progressing to *in vivo* and human applications, the latter has become very commonly used, because it has the long electron T_1 and narrow linewidth required to facilitate high polarization under the conditions of low temperature and moderate microwave power used in commercial instruments. A hydrophilic derivative, termed 'OX063' is widely used for pyruvate and similarly hydrophilic substrates [54]. A slightly modified version, known as 'AH111501' or simply 'EPA' (for Electron Paramagnetic Agent), is used in human studies due to the ease with which it can be removed at acidic pH by simple filtration [55]. Other substrate species, including those with hydrophobic properties, are best polarized by other trityl derivatives with less polar functional groups, such as the acidified Finland species successfully used for DNP of frozen Xenon [56].

However, a wide variety of other radicals, including those with similar electronic properties, may be suitable as well. Radicals derived from BDPA have been shown to behave similarly to the trityl family [54,57] and may be synthesized in a more straightforward manner [58]. Nitroxide radicals (eg., TEMPO [54]) are well suited to higher temperature DNP and hyperpolarization of hydrogen, including that of water [59–61]. A variety of biradical species have been explored, with the hope of maximizing the cross effect [62–64] with judicious chemical shift and coupling values. In pyruvate, there has even been exploration of metastable radical species induced in the frozen pyruvate sample itself by ultraviolet light [65]. Although as yet not consistent with the high polarization required for effective *in vivo* use, such a strategy is very appealing as no additional species are required for DNP.

A few other experimental details have been found to affect the signal level or utility of DNP-derived hyperpolarized species; we mention these here and leave the details to the referenced literature. Inclusion of small concentrations of Gd chelates in the sample has been shown to increase polarization substantially, perhaps through its effect on radical electron T_1 [66], although the potential for accelerated loss of polarization after dissolution must be considered [67]. The dependence of polarization on magnetic field and temperature have been explored as well, with the finding that lower temperature and higher field, while more technically challenging, yield generally superior results but require longer polarization times (typical small molecules can take anywhere from less than an hour to several hours to achieve maximum polarization at 1.4K and 3.3T) [68,69]. This is not found to be true in all systems, however. Finally, certain hyperpolarized nuclei, especially those neighboring a nitrogen atom, require transport at elevated magnetic field to avoid substantial loss of polarization [70].

3.2. Parahydrogen-Induced Polarization (PHIP)

Two alternate methods for liquid state sample hyperpolarization exist, termed Chemically Induced Dynamic Nuclear Polarization (CIDNP) [71,72] and Parahydrogen-Induced Polarization (PHIP) [73,74]. The former mechanism is based on a nuclear state dependence of the recombination rates of optically generated radicals; although it remains an active research area 50 years after its discovery, the level of polarization achieved and the diversity of target compounds are not yet such that *in vivo* or clinical molecular imaging applications can be considered. Compounds polarized using parahydrogen, on the other hand, have been used successfully in cell and animal studies [75–77]. Although recent efforts have been made to design an open-source PHIP polarizer [78], in general the adoption of PHIP techniques currently lags behind DNP because of the lack of commercial instruments, difficulties polarizing small metabolic compounds because of the required chemical reaction, and the lack of development in ensuring a pure and safe end-product. Nonetheless, the method has fundamental advantages with respect to production rate and cost that may justify more widespread use in the near future.

Parahydrogen is the nuclear singlet state of hydrogen gas. Although not hyperpolarized, the gas contains a large degree of nuclear spin order and is easily produced at modestly reduced temperature through contact with a microscopically heterogeneous magnetic substance [79,80]. After undergoing a catalyst-mediated, pairwise addition to an unsaturated substrate, this spin order often persists to a large degree. Subsequent application of a specialized NMR pulse sequence, analogous to the commonly-used INEPT polarization transfer technique [81], results in near-unity transfer of spin order to hyperpolarization of a nearby ^{13}C [82–85]. Alternately, in a process called field cycling, the transfer may be effected by reducing the magnetic field such that the chemical shift difference approximates the internuclear scalar couplings [86,87]. In either case, a level of polarization approaching or even exceeding that which is achievable using DNP [88] has led to the most detailed solution state hyperpolarized images produced to date [76,86].

Use in metabolic imaging, however, has been limited by the need for an unsaturated precursor of the agent of interest. In many cases this precursor does not exist or is not stable.

Even in cases where a suitable precursor exists (e.g., for pyruvate or acetate), care must be taken to ensure that only the desired hydrogenation end-product is produced [89–91]. Furthermore, the couplings between the nucleus to be hyperpolarized and the para proton pair must differ by an amount that is large compared to other couplings to the target nucleus [92]. These requirements can generally be addressed by careful choice of reaction conditions and catalyst and by replacing nearby hydrogen by deuterium (or as appropriate, ^{14}N by the less magnetic ^{15}N). Details of the experimental design and catalyst choice can be found in the referenced literature, but we note that most experimenters have made use of a homogeneous Rh-based bis-phosphene catalyst [77,88,93,89], a plunging liquid jet reactor [94] in an ambient parahydrogen atmosphere of several bar, and somewhat elevated temperature (to speed the hydrogenation reaction) [88,95,96]. More recently, PHIP using a heterogeneous phase catalyst—consisting of platinum nanoparticles capped with glutathione ligands—was demonstrated, enabling easier removal of the toxic catalyst, a necessity for clinical imaging [97].

Although not significantly metabolized *in vivo*, the first biomedical application of PHIP agents utilized hydroxyethyl propionate as an angiography or perfusion measurement agent [76,86]. Use in molecular imaging was first demonstrated using succinate or its esters [77,98,99], which are two-step hydrogenation products of acetylene dicarboxylate. Phospholactate has also been efficiently polarized using this method [89], although biological activity has not yet been shown.

Two important modifications to this basic scheme have been introduced recently, and each has the potential to dramatically improve the PHIP process' flexibility and its utility in imaging. Reineiri *et al.* introduced a method to overcome the lack of suitable precursors for pyruvate and acetate [90]. In this scheme, an allyl or vinyl ester of the target molecule is para-hydrogenated, and the polarization transfer sequence operates over the ester bond to hyperpolarize the labeled carboxyl carbon. Finally, the ester is hydrolyzed, yielding a mixture of the hyperpolarized target and ethyl or propyl alcohol. In addition to allowing PHIP of pyruvate, this recipe may be generally applicable to carboxylic acids, greatly increasing the biological uses of PHIP.

Another novel technique was recently introduced by Adams *et al.* [100], in which the spin order of parahydrogen is transferred to a target molecule during the residence time of a short-lived, catalyst-mediated complex. This method, termed 'NMR Signal Amplification by Reversible Exchange', or NMR-SABRE, has two significant advantages over traditional PHIP. First, the need for an unsaturated precursor is relaxed in that no permanent chemical modification of the target molecule occurs. This simplifies synthetic requirements while broadening the set of candidate compounds. Second, the same solution may be repolarized at will, subject only to availability of parahydrogen and catalyst spoiling. SABRE-polarized compounds have not yet been used *in vivo* for metabolic imaging, and isolation of the target compound remains a technical challenge. Nonetheless, these advantages (in addition to the low cost and ~1s polarization time characteristic of PHIP) suggest a variety of very useful applications in the near future.

4. Hyperpolarized MRI Acquisition Techniques

4.1. Hardware and Equipment

The most important development in the field of DNP was the development of a fast dissolution method that rapidly melted the frozen polarized sample with minimal loss of polarization into a liquid state that could then be quickly delivered to an animal or specimen in the imaging magnet [101,102]. This is accomplished by flushing the frozen sample with the dissolution solvent at high pressure (~10 bar) and temperature (~ 180 °C). The most commonly used DNP polarizer that uses this dissolution method is the commercially available Hypersense polarizer (Oxford Instruments, United Kingdom). Generally it can polarize up to ~200 µL of sample, limiting its use to studies in small animals. More recently, a larger commercially-available polarizer—SpinLab (General Electric)—was developed with a much greater throughput (4 simultaneous samples of volume greater than 1mL), enabling HP imaging in large animals and humans [103,104].

The gyromagnetic ratio of ^{13}C (γ_{C}) is approximately four times smaller than that of the proton. As such, the MR system must be equipped with a broadband RF amplifier to transmit and receive the NMR signal for multinuclear spectroscopy and imaging. This option is available for many clinical and preclinical scanners. Additionally, the MR system needs to be equipped with transmit/receive coils tuned to the proper frequency. For hyperpolarized ^{13}C studies, surface coils [105] have been commonly used for many applications including cardiac [106], brain [107] and subcutaneous tumor [108,109] imaging and spectroscopy. The key advantage of surface coils is their superior sensitivity over a small region-of-interest. However, surface coils suffer from poor B_1 homogeneity and sensitivity profiles. Alternatively, a setup consisting of a volume transmit, surface receive coil can be used for homogenous power transmission [103]. Volume transmit/receive coils offer superior field homogeneity on both transmit/receive ends and, as such, are well suited for imaging the larger regions of interest typically used in abdominal and thoracic applications [110,111]. For small animal and perfused cell applications, cryocoils have shown to improve the SNR, although the degree of improvement is difficult to determine with certainty [111,112]; imaging researchers have reported SNR increases of 4–7 at 9.4T, consistent with theoretical considerations under low-loading conditions [111]. Utility of the cryocoils is limited due to high cost and diminishing gains for larger animals and humans, in which body noise typically dominates.

4.2. Pulse Sequence and Imaging Considerations

Although hyperpolarization dramatically increases the initially available signal amplitudes, the MR data acquisition is highly affected by irreversible signal losses caused by T_1 relaxation back to thermal equilibrium and RF pulse excitations. This limits signal availability to only a brief period after introduction of the agent. Thus, the timing of the data acquisition is of key importance in order to obtain metabolite images with maximum signal-to-noise. For many hyperpolarized substrates, a 25-second delay from the start of the injection has been found to be appropriate as it approximately corresponds to the time of peak amplitude for the downstream metabolites (figure A) [113]. Accounting for signal dynamics is also important because for the image reconstruction it is typically assumed that

the longitudinal magnetization is in steady state for each excitation; departure from this condition can introduce image artifacts or blurring. Variable flip-angle strategies have been proposed to ameliorate this problem, in which the flip angle is increased with each excitation to compensate for the reduction in available longitudinal magnetization. The goal of this strategy is to maintain a constant transverse magnetization throughout the image acquisition [113]. This approach requires accurate calibration of the coil's RF power, which is typically done by placing a labeled ^{13}C phantom inside the coil next to the subject prior to administration of the hyperpolarized agent. Ideally, the coil's B_1 map should also be obtained and incorporated into the reconstruction to improve image fidelity [110,114]. This is particularly important when single-channel receive surface coils or multichannel phased-array receive coils are used for parallel imaging. More difficult to deal with is the potential for severe flow artifacts due to the presence of high concentrations of the administered hyperpolarized agent in the blood pool. Recently, a pair of symmetric flow-sensitizing gradients have been added to imaging pulse sequences in applications such as cardiac and abdominal imaging to suppress image artifacts caused by rapidly flowing blood and thereby improve the accuracy of the spatial information [115,116].

In order to address these challenges and limitations, many pulse sequences have been designed and implemented over the past few years that offer significant improvements with respect to standard gradient echo or spin echo schemes. This has been possible by exploiting the long T_2 and T_2^* relaxation times of ^{13}C species in almost all organs [76,117–120] as well as the sparse nature of the NMR spectrum in which chemical shifts of the ^{13}C species of interest span a wide range of the NMR spectrum ($\sim 100\text{ppm}$) [121]. The most desirable imaging pulse sequences achieve superior spatial coverage as well as high temporal resolution while providing three dimensional dynamic spectroscopic data for accurate and meaningful measurement of cellularity and metabolic pathways.

4.2.1. Global and Local Spectroscopy—The most basic pulse sequence used for hyperpolarized ^{13}C spectroscopy is the nonselective pulse-and-acquire sequence used to assess the time-course of the agent and metabolites within the detection volume of the RF coil [122]. This pulse sequence is ideal for applications such as the study of perfused organs and cells [106,123,124] where changes in flux and pool size of metabolites are being investigated. Spatial selectivity can be incorporated by adding slice selection over a region of interest. This method is useful for organ-specific applications [125,126] as well as tumor xenografts [108,109]. Other methods with 3D localization have been subsequently introduced including a Point RESolved Spectroscopy (PRESS) pulse sequence with a slice-selective 90° RF excitation pulse followed by two refocusing pulse pairs along the other axes [118].

4.2.2. Multi-echo Approaches to Chemical Shift Imaging—Single-point chemical shift imaging (CSI) that uses one RF excitation per sampled FID has been used most frequently for generating maps of individual metabolites [122]. However, it suffers from a long acquisition time (typically on the order of 10–20 s for a single slice with a 16×16 matrix size and 256 RF excitations). In hyperpolarized imaging, this necessitates the use of a low flip-angle which carries a significant signal-to-noise penalty. However, due to its

simplicity and availability on most clinical and preclinical MR scanners, single-point CSI sequence is still a popular pulse sequence (figure B) [104]. Furthermore, its simplicity makes CSI relatively immune to artifacts; thus, even if higher resolutions or faster acquisitions can be achieved using other methods, single-point CSI can yield well-understood reference images for validation.

Alternatively, the long T_2 relaxation time of ^{13}C allows for a long readout duration, during which multiple echoes can be generated and spatially or spectrally encoded to reduce the acquisition time. Echo-planar Spectroscopy Imaging (EPSI) sequences [127] have been extensively used to image hyperpolarized ^{13}C metabolites. In these schemes, each readout is used to spatially encode the data while the accumulated phase between acquisitions associated with the chemical shift, which is not refocused by symmetric gradients, is used to encode the frequency information. Spatial encoding in this manner reduces the number of excitations significantly when compared to the single-point CSI sequence. For instance, a 16×16 single-slice image can be acquired using only 16 RF excitations, drastically reducing the total imaging time and permitting the application of higher flip angles. These improvements have allowed practical use of a 3D implementation of the sequence [128]. Further improvements to spectral information quality were found when using a double spin-echo implementation of EPSI [129]. This modified sequence has been used in many studies, including the first human studies of prostate cancer [103]. Multi-echo spiral readout trajectories with multiple interleaves have also been demonstrated for dynamic two-dimensional [130,131] and volumetric imaging [107]. When compared to the Cartesian echo-planar spectroscopic read-out trajectories, spatial encoding can be performed more efficiently using a spiral readout trajectory. Additionally, spiral trajectories are less sensitive to motion artifacts, making them very robust for cardiac applications. Recently a concentric ring readout trajectory was introduced as another alternative to the echo-planar readout trajectory [132]. This scheme can be easily combined with parallel imaging for further acceleration, while offering improved acquisition speed and robustness to flow artifacts with respect to the echo-planar readout.

Instead of using phase evolution over multiple echoes to encode chemical shift information, it is also possible to use a least-square algorithm to iteratively decompose different chemical shift components of the NMR spectrum. This method, also known as Iterative Decomposition of water and fat with echo Asymmetry and Least square estimation (IDEAL) obtains spectral information by shifting the echo time (TE) [133]. The method is used extensively for selective imaging of water and fat in proton MRI; however it can be extended to a larger number of resonance frequencies if at least as many echoes are acquired as there are spectral peaks [134]. The reconstruction requires fewer echoes than an EPSI sequence but it demands prior knowledge of the location of the peaks in the NMR spectrum and a B_0 map to successfully separate the individual frequency components. The method has been successfully used with Cartesian trajectory gradient echo sequences for two-dimensional [135] and three-dimensional [136] single time-point imaging, spiral [137] and Echo-Planar Imaging (EPI) readout trajectories [138] for 2D dynamic imaging, and as a single-shot spin-echo sequence with multiple Cartesian readouts [122] for high resolution two-dimensional imaging. The latter approach showed an over 30-fold reduction in imaging time compared to a conventional single-point CSI sequence.

4.2.3. Spatial-Spectral Excitation for Chemical Shift Imaging—A completely different approach is to exploit a sufficiently large chemical shift separation between peaks to selectively excite individual metabolites. The advantage of this approach is that it eliminates the need to spectrally encode the NMR signal, thereby reducing the total imaging time and potentially offering higher signal-to-noise [138]. It can also facilitate efficient time-resolved imaging [128] in dynamic applications and in studies requiring triggering such as cardiac imaging [114]. Spatial spectral (SPSP) excitation is achieved by using composite pulses consisting of multiple lobes of short RF excitations accompanied by time-varying gradients (typically an echo planar gradient waveform). Spatial selectivity is determined by the shape of each RF pulse lobe, while spectral selectivity depends on the envelope of all the lobes. With *a priori* knowledge of the underlying spectrum, SPSP RF pulses can be optimized for hyperpolarized ^{13}C MRI to achieve superior spectral selectivity and minimal duration. Multiband RF pulses can also be designed to excite each metabolite with a different flip angle. This technique increases both signal longevity and metabolite SNR [139–142]. SPSP RF pulses can be accompanied by the appropriate EPI readout strategy to support two-dimensional [138,143] and three-dimensional [128,144] time-resolved imaging, or by a spiral readout for multiline cardiac imaging [114], three-dimensional imaging [145] and time-resolved three-dimensional imaging of tumors [146]. The latter sequence generates high-resolution metabolite images with a single SPSP excitation pulse combined with a three-dimensional partial k-space spiral readout over multiple echoes generated via double spin-echoes, yielding a 2-second temporal resolution. The pulse sequence diagram is shown in figure C. Such RF pulses can be used for outer volume suppression in *in vivo* organ-specific spectroscopy [147] or with multi-echo approaches, such as the EPSI sequence for dynamic imaging [138,139].

4.2.4. Other Approaches to Chemical Shift Imaging—Given the large chemical shift separation between the ^{13}C species, the chemical shift displacement artifact (CSDA) can be exploited to acquire images of the individual metabolites in a single acquisition. Several studies have demonstrated this approach for gradient echo sequences with Cartesian readout trajectories [148,149] in which the chemical shift separation between all peaks relative to the acquisition bandwidth is sufficiently large for complete spatial separation of their corresponding images. Alternatively, the severe smearing artifact of off-resonance peaks when using radial [150] and spiral [151] readout trajectories can be exploited to reconstruct images of a single resonance. While this approach provides images of all metabolites simultaneously, it can suffer from blurring if the T_2^* is shorter than the duration of the readout gradient. Thus, the approach constitutes a tradeoff; progressively lower bandwidth provides superior peak separation, but requires longer gradient durations with the potential for increased blurring.

CSDA can also be used along the slice selection orientation by using a small bandwidth frequency selective RF pulse excitation such that the displaced excitation band of the non-desired metabolites shifts outside of the coil [152]. This approach has advantages over SPSP RF pulses due its simplicity, but provides limited slice selection accuracy and the geometry of the coil and the subject size limits the imaging parameters.

4.2.5. Perfusion Imaging—Imaging of perfusion (non-metabolized) agents is generally much less sophisticated than chemical shift imaging because it does not require spectral encoding. As such, single-shot Fast Spin Echo (FSE) [120] or balanced Stead-State Free Precession (bSSFP) sequences are capable of producing high-resolution multiline or three-dimensional time-resolved images for the quantification of organ perfusion and blood flow [76,117,119,148,153]. Multivalent perfusion imaging [154] or simultaneous perfusion and metabolic imaging [115,155] can be performed as well, as long as some form of spectral encoding is included as discussed above.

4.2.6. Accelerated Imaging—Parallel imaging has been shown to yield significant reductions in acquisition speed and generally does not carry the signal-to-noise penalty that is observed in traditional ^1H MRI [132,156,157]. Another approach is to implement compressed sensing into the imaging sequence to exploit the sparsity of the NMR spectrum. Compressed sensing has been combined with single-point CSI [158], three-dimensional EPI with SPSP excitation [159], EPSI and multiband SPSP excitation [160]. Up to a 7-fold reduction in acquisition time has been achieved, allowing efficient time-resolved, three-dimensional imaging.

4.3. Quantification Methods

Quantification of HP data is not straightforward because, unlike with other modalities, the signal is not directly proportional to the concentration of the imaging agent or metabolite, and methods for absolute calibration have not been developed. Most studies to date have evaluated the data using one of two divergent methods: through quantification of signal ratios or by deriving fitting parameters from a kinetic model. Relative quantification typically reports the metabolite signal (area under the curve) normalized by either the delivered HP probe's signal, or the total carbon metabolite signal [161–164]. The advantage of this method is that it is trivial to perform, but it limits a direct comparison between studies because the normalization does not explicitly account for the delivered probe concentration, transport mechanics, T_1 or RF-induced relaxation or other sources of signal variation.

The second approach generates kinetic parameters (enzyme kinetic rate constants) by fitting HP data to a set of differential equations that model the HP label exchange between the injected probe and converted metabolites. The most commonly used model is the two-site exchange model [108], although there are a number of other models and analysis techniques that vary in complexity [165–167]. Deriving parameters from a fitted model has the advantage that it reflects a physical value (i.e., the rate constants) that can be compared to other studies. However, the generalizability of the results is limited by the constraints of the chosen model, and thus are in most cases more accurately described as apparent rate constants. Currently, there is no consensus on the ideal model that is applicable to all HP MRI/NMR studies, but development of such a model is necessitated by the current push towards clinical imaging [24].

5. Hyperpolarized Probes Used for Metabolic Studies

A number of different probes have been successfully polarized over the years for their use in biological studies. As described in sections 3.2 and 3.3, the ideal probes are small, mobile

molecules that have a relatively long T_1 relaxation time. Furthermore, a probe that is used for metabolic or functional imaging must also be metabolically relevant in the organ or tissue of interest. This section highlights the HP ^{13}C probes that have been most actively used in metabolic studies due to their ubiquity in the metabolism of many different tissues, or their specific applicability to assessing the tissue's state.

5.1. $[1-^{13}\text{C}]$ Pyruvate

As alluded to in the earlier sections, the most widely used hyperpolarized DNP probe is $[1-^{13}\text{C}]$ pyruvate. Its physical characteristics make it an ideal probe for DNP. The labeled carbon is a carbonyl that is three bonds away from other magnetic species, minimizing dipolar coupling. As a small molecule, it also has low correlation times (τ_C), resulting in longer T_1 than larger molecules. As a carboxylate, it also has high aqueous solubility. These favorable physical properties enable high polarization (up to 60% polarization reported) and long T_1 of 40–60s [24,168]. Moreover, biologically, pyruvate is an intermediate in several key metabolic pathways. Together, these physical and biological properties make $[1-^{13}\text{C}]$ pyruvate perhaps the most attractive HP imaging probe.

Pyruvate is a downstream product of glycolysis, and serves as a key intermediate in several metabolic pathways (figure D). It rapidly and reversibly catalyzes to lactate via the enzyme lactate dehydrogenase (LDH) [169]. Physiologically, conversion of pyruvate to lactate is determined by LDH activity and the ratio of NADH to NAD⁺ [170]. However, this does not imply that the HP $[1-^{13}\text{C}]$ lactate signal detected after HP $[1-^{13}\text{C}]$ pyruvate administration represents net conversion of the substrate. In fact, the observed metabolite signal is largely representative of label exchange between endogenous lactate and injected HP $[1-^{13}\text{C}]$ pyruvate [23]. As such, the observed signal reflects the endogenous lactate pool size and the redox state of the system. Many external perturbations or pathologies change the lactate pool, thereby allowing HP pyruvate to be used as a biomarker in many different pathologies, including acute injury, inflammation, or cancer [104,124,171,172].

Pyruvate is also reversibly catalyzed to the amino acid alanine via the enzyme alanine transaminase (ALT). In most tissues, HP $[1-^{13}\text{C}]$ alanine signal is substantially less than that of $[1-^{13}\text{C}]$ lactate, due to the lower activity of ALT, or the lower alanine pool size, compared to LDH and lactate, respectively [161,173,174]. However, HP $[1-^{13}\text{C}]$ pyruvate's conversion to HP $[1-^{13}\text{C}]$ alanine is a useful biomarker for tissues that are heavily involved in alanine metabolism, particularly the liver and skeletal muscles [122,175].

The third most common fate of HP $[1-^{13}\text{C}]$ pyruvate is its conversion to acetyl coenzyme A (acetyl-CoA) and $^{13}\text{CO}_2$ via pyruvate dehydrogenase (PDH). The $^{13}\text{CO}_2$ is then rapidly converted to $[^{13}\text{C}]$ bicarbonate via the enzyme carbonic anhydrase (CA) [176]. Although the $[^{13}\text{C}]$ bicarbonate SNR is too low to be easily quantifiable in most healthy tissues, it is prominent in the heart, which is much more energetically demanding than most other organs [177]. Because the acetyl-CoA is not labeled, its fate—typically incorporation into the tricarboxylic acid (TCA) cycle via the pyruvate dehydrogenase complex (PDC) or conversion to acetylcarnitine (ALCAR)—cannot be determined. Nonetheless, the use of HP $[1-^{13}\text{C}]$ pyruvate to study TCA cycle activity is possible in the liver and other tissues expressing pyruvate carboxylase (PC). This enzyme irreversibly catalyzes pyruvate to

oxaloacetate (OAA), which can then be used as a substrate for gluconeogenesis or converted to other TCA cycle intermediates [178].

Due to the versatility of [1-¹³C]pyruvate, it can be used to study a wide variety of metabolic perturbations in many tissues. The most notable difficulty is that the signal-to-noise is too low to be useful in true tracer applications. Thus, metabolic perturbations by superphysiological agent concentrations (mM and above) must be considered. In some circumstances, this may limit sensitivity to disease and the relevance of quantitative physiological measurements [167,179].

5.2. [2-¹³C]Pyruvate

HP [2-¹³C]pyruvate is used much less frequently than HP [1-¹³C]pyruvate. As the 2-carbon is labeled, the closest magnetic interaction is two bonds away. This results in a slightly lower T₁ than that of the 1-carbon [180]. Nonetheless, HP [2-¹³C]pyruvate imaging is much better suited for studying oxidative phosphorylation. Unlike [1-¹³C]pyruvate, the HP carbon is found on acetyl-CoA after PDH catalysis, so HP [2-¹³C]pyruvate can be used to study PDC flux, fatty acid oxidation (acetoacetate, acetylcarnitine), and conversion to TCA cycle intermediates or downstream products (e.g., [1-¹³C]citrate and [5-¹³C]glutamate) [181,182]. This is particularly useful for studying cancers that disrupt oxidative phosphorylation. Nevertheless, until recently a limited number of studies have been performed using HP [2-¹³C]pyruvate because of the relatively low oxidative phosphorylation activity in many tissues.

5.3. [1-¹³C]Acetate

HP [1-¹³C]acetate is typically used as a probe to study TCA flux and fatty acid oxidation. The enzyme acetyl-CoA synthase (ACS) rapidly converts free acetate into acetyl-CoA. The acetyl-CoA can then either enter the TCA cycle and its conversion to TCA intermediates can be followed, or it can be reversibly converted to acetylcarnitine (ALCAR) via the enzyme carnitine acetyltransferase (CAT), depending on the energy needs of the tissue [183]. As a probe, HP [1-¹³C]acetate bypasses the PDH complex, so unlike pyruvate, it provides a direct measurement of TCA flux. HP [1-¹³C]acetate studies are useful in tissues that prioritize fatty-acid metabolism as opposed to glucose metabolism, and consequently most studies have been done in the heart or skeletal muscles [184,185].

5.4. [1-¹³C]Alanine

HP [1-¹³C]alanine has also been used as an alternate probe for studying pyruvate metabolism. Upon injection, HP [1-¹³C]alanine is converted to HP [1-¹³C]pyruvate via ALT, which can then be converted to HP [1-¹³C]lactate. Using HP [1-¹³C]alanine is advantageous as it allows relative measurements of endogenous pyruvate and lactate pool size [186] without the analytical difficulties arising from the large extracellular pyruvate signal. Alanine's metabolism varies by organ, but ALT is especially active in the muscle and liver, which play a key role in the Cahill cycle that shuttles alanine and glucose between the two organs [187]. A recent study has also used a modified alanine moiety, HP [1-¹³C]alanine-NH₂, to specifically target aminopeptidase N, an enzyme that is upregulated in tumor angiogenesis [188].

5.5. [^{13}C]Urea

[^{13}C]urea has also been used as a HP probe. Unlike most other probes, urea is metabolically inactive. Instead, it has been used as a contrast agent or a perfusion agent. It is a promising alternative to traditional contrast agents (such as gadolinium) as it offers the unique advantages of high contrast (no background signal), more straightforward quantification, and minimal toxicity [115,119,120].

5.6. [$1\text{-}^{13}\text{C}$]Dehydroascorbic Acid

Dehydroascorbic acid (DHA) is the oxidized form of ascorbic acid (vitamin C); the relative concentration of the two can provide information on the intracellular redox state [189]. DHA uptake varies wildly by tissue, so it cannot be used as a universal probe [190,191]. Unlike the other smaller metabolites, [$1\text{-}^{13}\text{C}$]DHA also has a comparatively short T_1 (~20s) at high fields (9.4–11T), so imaging at those fields requires fast imaging sequences; however, the reported T_1 at clinical MRI fields is much higher (~57s), making studies at the lower field much more feasible [192,193].

5.7. [^{13}C]Succinate/[$1\text{-}^{13}\text{C}$]Diethyl Succinate [$1,4\text{-}^{13}\text{C}_2$]Diethyl Succinate

Succinate, a TCA cycle intermediate, can be reversibly catalyzed to either succinyl-CoA via the enzyme succinyl-CoA synthetase, or to fumarate via the enzyme succinate dehydrogenase [194,195]. The ability to image the fate of succinate would provide insights into many pathologies that affect oxidative phosphorylation [196,197], especially in tissues where no TCA intermediates are detected with HP [$1\text{-}^{13}\text{C}$] or [$2\text{-}^{13}\text{C}$]pyruvate. [$1\text{-}^{13}\text{C}$] or [$1,4\text{-}^{13}\text{C}_2$]succinate have not been successfully polarized using DNP. However, [$1\text{-}^{13}\text{C}$]succinate has been successfully polarized with PHIP using [$1\text{-}^{13}\text{C}$, $2,3\text{-}^2\text{H}_2$] fumaric acid and [$1\text{-}^{13}\text{C}$]acetylenedicarboxylate, with reported T_1 values of 27s (at 4.7T) and ~6s (at 1.5T), respectively [98,99,198]. Other attempts to polarize succinate have used ethyl groups to protect the terminal carbonyl groups. The resultant diethyl succinate has been polarized via both PHIP and DNP, with a reported T_1 of ~38s at lower field strengths (3 and 4.7T) [77,199].

5.8. Other Hyperpolarized Metabolites

There are other less extensively studied molecules that have also been successfully polarized. One promising probe is [$1\text{-}^{13}\text{C}$]bicarbonate, which rapidly converts into HP $^{13}\text{CO}_2$ via the enzyme carbonic anhydrase [149,200]. The ratio of bicarbonate-to-carbon dioxide can be used to derive tissue pH, which is a powerful biomarker for both acute and chronic pathologies [201,202]. [$\text{U-}^{13}\text{C}_6$, $\text{U-}^2\text{H}_7$]glucose, which lies metabolically upstream of pyruvate, has also been successfully polarized, but its *in vivo* use is hindered by its short T_1 (about 10–13s) and complex spectrum [203,204]. For an exhaustive list of the molecules that have been successfully polarized, please refer to the comprehensive review by Keshari and Wilson [121].

6. Hyperpolarized Studies by Organ System

A number of excellent reviews on the state of hyperpolarized DNP have been written in the past, each focusing on different aspects of the field. The review by Barnes *et al.* [48] focuses

on the physics of DNP, those by Karlsson *et al.* [52], and Keshari and Wilson [121] on the chemistry of DNP probes. Reviews have also been written with a particular biological or pathological focus, e.g., on cancer [205] or neurology [99]. However, none has described how HP studies have varied according to the different tissue or organ systems they have been used to assess. As such, this section summarizes the major HP studies by organ or tissue to characterize their metabolic profile for a given set of HP probes. This survey will also highlight the unique challenges that each tissue poses for molecular imaging. For example, probe delivery to the brain is limited by the blood brain-barrier, whereas the lung presents the challenge of the air-tissue interface. The section describes *ex vivo*, *in vivo*, and *in vitro* studies of the heart, liver, brain, prostate, kidney, lung, muscle, and breast.

6.1. Heart

Proton MRI is used quite often for structural cardiac imaging. In addition to its high spatial resolution, MRI's sensitivity to flow and ability to freeze motion during the cardiac cycle make it suitable for answering many clinical questions. Other nuclei, such as ^{31}P , have been used to obtain metabolic information about the heart in healthy and diseased states; however, given their low gyromagnetic ratio and metabolite concentration, *in vivo* application is fairly limited.

Hyperpolarized ^{13}C MRI provides a new opportunity for real-time metabolic imaging of the heart. A number of isolated perfused and *in vivo* heart studies of small and large animals have been carried out to characterize the metabolic profile of the healthy heart using HP agents. Unlike most other tissues for which HP lactate labeling or HP alanine labeling is primarily observed, significant conversion of HP $[1-^{13}\text{C}]$ pyruvate to HP bicarbonate has been detected in heart [114,161,177,206]. In healthy hearts, the detected HP bicarbonate reflects PDH flux, but not necessarily flux through the TCA cycle [177]. In the fasted state, when the heart switches to fatty acid oxidation as its primary energy source, a decrease in HP bicarbonate signal has also been observed [161,179,206]. Adding butyrate or octanoate (even-chained fatty acids) to the perfusate has produced a similar decrease in HP bicarbonate signal; moreover, the TCA flux measured via tissue extracts was unchanged, providing direct evidence for decreased PDH flux in the presence of fatty acids [177,207]. Adding dichloroacetate (DCA), a well-known pyruvate dehydrogenase kinase (PDK) inhibitor, promotes PDH flux, yielding a stronger HP bicarbonate signal, as expected [183,206,208,209]. Adding dobutamine, a selective β -agonist, increased PDH flux as well as TCA flux, as determined by HP $[2-^{13}\text{C}]$ pyruvate MR. This demonstrates that the different HP probes can be used to assess different types of stress on cardiac tissue [183].

Given that the heart can derive the acetyl-CoA needed for the TCA cycle from either pyruvate decarboxylation or β -oxidation, a number of studies have focused on using $[1-^{13}\text{C}]$ acetate's conversion to downstream TCA intermediates or reversible conversion to acetylcarnitine as another HP biomarker to characterize cardiac metabolism [185,210,211]. These *in vivo* studies in rats and pigs characterized the TCA flux using ratiometric modeling of HP acetate, acetylcarnitine, and citrate signals in healthy animals. More recently, HP $[1-^{13}\text{C}]$ butyrate was successfully used to characterize TCA intermediates in fed and fasted

isolated perfused rat hearts and confirmed previous findings that the presence of fatty acids limits PDH flux without affecting TCA flux [207].

These HP markers have also been used to characterize a number of cardiac pathologies. HP [1-¹³C]pyruvate studies of ischemia-reperfusion in rats and pigs have shown a decrease in HP bicarbonate signal and an increase in HP lactate signal immediately after ischemia [212–214]. Partial recovery to baseline was observed 15 minutes post-reperfusion [212], suggesting that HP [1-¹³C]pyruvate could serve as a marker for ischemia-reperfusion injury. The ratio of HP bicarbonate to HP CO₂ was also used to characterize this injury, and it was found that post-ischemia pH was about 7.12, compared to 7.20 in healthy rats [214]. An HP [2-¹³C]pyruvate MR study yielded complementary findings that showed an increase in HP [2-¹³C]lactate labeling, and a decrease in [1-¹³C]acetylcarnitine, [1-¹³C]citrate, and [5-¹³C]glutamate, in isolated perfused ischemic rat hearts [214].

In vivo HP [1-¹³C]pyruvate NMR also showed a decrease in HP bicarbonate production in a streptozotocin-induced type 1 diabetes model in rats [161]. These results confirm the well-established finding that the onset of type 1 diabetes decreases glucose oxidation and PDH flux [215].

Although earlier studies were performed in perfused hearts and small animals, the adoption of modern pulse sequences and fast sequences has resulted in more *in vivo* studies, as well as studies in larger animals where the signal emanating from the heart chambers and the myocardium can be resolved. In addition to confirming the results from the previous studies, these *in vivo* studies have shown the particular region of the myocardium tissue from which the bicarbonate and lactate signals originate [115,144].

6.2. Liver

The liver is the largest gland and the most metabolically active organ in the human body. Unlike most organs, it is heavily involved in whole-body metabolism [216]. As such, a diverse set of metabolic pathways and HP probes has been studied in the liver.

The liver is largely responsible for gluconeogenesis, the process by which glucose is synthesized from lactate and alanine (as part of the Cori and Cahill cycles, respectively) when blood glucose level is low [187,217]. An HP [1-¹³C]pyruvate study of the rat liver in the fed (high blood glucose) and fasted (low blood glucose) states demonstrated this process *in vivo* by detecting the expected decrease in alanine pool size via a decrease in HP alanine labeling in the fasted state [186]. The liver is also one of the few organs that expresses pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEPCK), allowing conversion of HP [1-¹³C]pyruvate to [1-¹³C]oxaloacetate and additional downstream metabolites ([1-¹³C]malate, [4-¹³C]malate and [1-¹³C]aspartate) (figure D) [178]. Changes in the PC flux and PEPCK flux after [1-¹³C]pyruvate injections in a type 2 diabetic mouse model demonstrated that this pathway could be exploited for longitudinal monitoring of diabetes development [218].

Several other studies have focused on tissue non-specific metabolic pathways and how they differ in the liver. Using HP [1-¹³C]acetate injections, the acetylcarnitine pool size was

shown to be lower than that in the heart [219]. HP [1-¹³C]alanine injection has been used to estimate the intracellular ratio of the pyruvate and lactate pools [186]. This technique is feasible in the liver, as opposed to most other organs, due to its relatively high ALT flux. Ethanol administration resulted in an increased lactate production rate in rat liver, most likely due to increased NADH availability after ethanol breakdown [126].

A large number of hepatic studies have focused on tumor imaging. Hepatocellular carcinoma (HCC) is an aggressive primary tumor with extremely high morbidity and mortality [220] in which glutamine metabolism plays an important role. The conversion of HP [5-¹³C]glutamine to HP glutamate was shown can be a possible biomarker for cell proliferation in human hepatoma (HepG2) cells [221]. Other studies have shown that the conversion rates and the T₂ relaxation times of alanine and lactate from HP pyruvate are higher in HCC tumors than in normal liver, suggesting a potential set of biomarkers for tumor characterization [118,222–224]. Another *in vivo* study found that transcatheter arterial embolization (TAE) of HCC in rats lead to increased conversion of HP [1,4-¹³C₂]fumarate to HP [1,4-¹³C₂]malate due to extracellular fumarase after necrosis, as well as a decrease in HP [¹³C,¹⁵N₂]urea and HP [1-¹³C]pyruvate signal, most likely due to decreased blood perfusion [225]. The conversion of HP [1-¹³C]α-ketoisocaproate (KIC) to HP leucine was also shown to be associated with branched-chain aminotransferase (BCAT) expression, an enzyme that is more strongly expressed in HCC cells, compared to normal liver tissue [223].

6.3. Brain

Many brain abnormalities can be clinically diagnosed with the structural information provided by conventional ¹H MRI, including multiple sclerosis, Alzheimer's disease, epilepsy, acute ischemic stroke and some high-grade brain tumors [137,226–230]. ¹H, ³¹P or ¹³C-based MR spectroscopy (MRS), on the other hand, can be used to diagnose bioenergetics-linked disorders, including early signs of cancer [231–233]. In particular, the Warburg effect—i.e., increased aerobic glycolysis and lactic acid fermentation—can be exploited as a biomarker for tumor detection [234].

While the clinical significance of ¹H-MRS in the detection of lactate in brain tumors is well established, *in vivo* imaging is limited by long acquisition times and complex spectra that are difficult to interpret [235–239]. Furthermore, exogenous contrast enhanced imaging is limited to small molecules due to the blood brain barrier, which metabolic substances must pass through for any neuronal or glial metabolism can occur [240,241]. FDG PET has been clinically used with a high sensitivity in diagnosis of cancer in many organs [242,243], and the agent does pass through the blood brain barrier, but it lacks specificity in brain due to the intrinsic high FDG uptake in normal surrounding grey matter and low uptake in some tumor types [244,245]. HP ¹³C imaging overcomes many of these limitations, and as such has been demonstrated to be a viable brain imaging technique.

The earliest *in vivo* HP [1-¹³C]pyruvate study in rat brains with human glioblastoma xenografts showed a higher lactate-to-pyruvate ratio in tumor regions compared to normal brain tissue [238]. Furthermore, the two immunochemically distinct tumor cell lines (U-251 MG and U-87 MG) used in the study had different HP signal characteristics. This approach was extended to study the response of chemotherapy (Temozolomide) and it was found that

a decrease in lactate-to-pyruvate ratio (day 1) preceded a decrease in tumor size (day 5 or 6), suggesting that this technique can be used to monitor tumor response to therapy [246]. This was further corroborated by a study using a PI3K signaling inhibitor (Everolimus) to treat implanted GBM that showed changes in HP lactate-to-pyruvate ratio about 7 days before any changes in tumor size or growth were detected [247]. HP [1-¹³C]pyruvate imaging has also been used to study C6 glioma, showing a higher lactate-to-bicarbonate ratio, higher lactate, and lower bicarbonate than healthy rat brains [248]. Radiotherapy in the same model showed a decrease in lactate-to-pyruvate ratio 96 hours post irradiation [249]. These studies suggest that this technology can be an effective alternative for brain cancer diagnosis and management. In that vein, [1-¹³C]pyruvate imaging was conducted on healthy nonhuman primate brains to study the translational feasibility of this methodology [125].

The past decade of HP brain imaging has also seen the use of several ¹³C contrast agents, both DNP- and PHIP-derived, for imaging different metabolic pathways. One early attempt at brain imaging used the PHIP technique for HP ¹³C–succinate production and cancer imaging, but the resulting image had insufficient spatial resolution to distinguish the tumor [98]. Later, HP diethyl succinate PHIP was introduced for real-time hyperpolarization overcoming the poor transport of HP ¹³C succinate across biological membranes [77]. Once decarboxylated inside the cell, entry into the TCA cycle is rapid. In a similar manner, [1-¹³C] DNP-polarized ethyl pyruvate traverses the blood brain barrier than pyruvate [250] and is metabolized post-deesterification. Finally, a simultaneous multi-agent (co-hyperpolarized [¹³C]urea, [¹³C]hydroxy-methyl cyclopropane, and [¹³C]t-butanol) was introduced for ¹³C perfusion imaging [154].

6.4. Prostate

Hyperpolarized ¹³C methods have been used extensively to study the prostate and in particular prostate cancer. Conventional clinical methods rely on prostate specific antigen (PSA) for screening and biopsies for diagnosis. In 2011, the United States Preventive Services Task Force (USPTF) recommended against PSA screening as the risk may outweigh the benefits [251]. Biopsies are often difficult to justify as well, largely due to their invasiveness and the difficulty of accurate sampling in the highly heterogeneous gland. ¹H high resolution magic angle spinning (HRMAS) spectroscopy and ¹H MRSI studies have shown that changes in choline-containing compounds, polyamines, and citrates can be used to improve assessment of cancer location in prostates, but these compounds cannot be easily identified using HP ¹³C NMR/MRI methods [252]. ¹H HRMAS also showed elevated lactate and alanine concentrations in human biopsy samples of patients with prostate cancer [253]. *In vivo* imaging of these metabolites using ¹H MRSI is challenging because their resonances lie close to the lipid resonances surrounding the prostate tissue. In contrast, HP 1-¹³C pyruvate imaging is an ideal tool for real-time measurements of lactate and alanine as potential biomarkers for prostate cancer.

A number of HP 1-¹³C pyruvate studies have been conducted that demonstrate the technique's feasibility to evaluate prostate cancer. The most widely used animal model is the transgenic adenocarcinoma of mouse prostate (TRAMP) model [254], which has shown increased HP lactate labeling in multiple *in vivo* studies [172,173]. The lactate signal (or

lactate to pyruvate ratio) was also correlated with increasing tumor grade [255]. It is important to note that due to the small size and multi-lobar structure of a normal mouse prostate, the tumor lactate measurements in these studies were compared to lactate measurements from either the kidney or liver (unchanged in control and TRAMP cohorts). All of these findings were confirmed using emerging imaging techniques (section 4), which further showed the heterogeneity of the tumor, especially in relationship to disease progression [255,256]. Other TRAMP studies also demonstrated the use of HP [1-¹³C]pyruvate spectroscopy to extract kinetic parameters for this disease; however, it was found that the fitted rate constants were heavily dose-dependent in the simplistic model used [165].

The findings from the *in vivo* TRAMP model have been translated to *ex vivo* and *in vivo* human studies. *Ex vivo* HP [1-¹³C]pyruvate studies using tissue slice culture (TSC) cells derived from radical prostatectomy specimens showed different metabolic ratios compared to patient biopsies. However, the trend in changes between the benign and cancerous tissue were similar in all the studies, i.e., increasing lactate with increasing cancer grade [257]. In 2013, the first in-man HP [1-¹³C]pyruvate study was performed in 31 patients with prostate cancer [103]. Being a phase 1 trial, its primary goal was to study the dose dependent effects of delivering HP [1-¹³C]pyruvate. The study confirmed the safety of the technique at the highest delivered dose of 0.43 mL/kg (of 230 mM agent), and demonstrated increased HP lactate/pyruvate in biopsy-confirmed regions of cancer, paving the way for further human studies (figure E).

Although most work in the prostate has used HP [1-¹³C]pyruvate, other HP agents have also been used to study prostate cancer. HP [2-¹³C]fructose's conversion to fructose-6-phosphate via hexokinase was found to increase in the tumor region [258]. DHA was also used in the TRAMP model, and the ratio of DHA to DHA plus ascorbate was found to increase in the late stage of the disease [189].

6.5. Kidney

Over the last decade, hyperpolarized ¹³C NMR/MRI has been used extensively to study changes in blood flow and metabolism in the kidneys and renal system. The kidneys receive approximately 20% of the cardiac output, making high ¹³C SNR easily achievable following intravenous administration of a hyperpolarized bolus.

The metabolism of healthy kidneys has been characterized using hyperpolarized [1-¹³C]pyruvate, in a manner similar to other organ systems. An *in vivo* rat study demonstrated significant conversion of HP pyruvate to both HP lactate and HP bicarbonate in the kidneys, while there was minimal conversion to alanine [259]. In another study, when pigs were given an oral sucrose load, it was found that the lactate to pyruvate ratio correlated with the blood glucose level, while all other metabolites of pyruvate remained unchanged [104].

In contrast to the metabolically active pyruvate, the metabolically inert [¹³C]urea has been used to characterize blood flow and other functional parameters in the kidneys. An *in vivo* rat study using [¹³C]urea showed more than a three-fold difference in renal blood flow

compared to hepatic blood flow [260]. Another *in vivo* rat study demonstrated that the T_2 of [^{13}C , ^{15}N]urea in kidneys was greater than ten seconds, as opposed to 1.3 seconds in blood, likely due to deoxyhemoglobin-induced paramagnetic relaxation in blood [119]. Finally, an *in vivo* rat study demonstrated more rapid medullary [^{13}C]urea enhancement in the antidiuretic state, suggesting that [^{13}C]urea could be used as a marker for urea transporter UT-A1 activity [261].

Hyperpolarized imaging techniques have also been used to characterize disease models in kidneys. Renal cell carcinomas (RCCs) are a broad group of tumors affecting the kidney that have been studied in some detail using hyperpolarized ^{13}C . *In vitro* HP [$1\text{-}^{13}\text{C}$]pyruvate studies of two different RCC cell lines (UOK262 and UMRC6) and healthy proximal tubule epithelial (HK-2) cells have shown that the observed pyruvate to lactate ratio and the ratio of HP intracellular lactate to extracellular lactate (efflux ratio) can be used to distinguish the three different cell lines from each other [262,263]. Furthermore, the HP lactate efflux ratio strongly correlates with MCT4 mRNA expression, suggesting its potential use as a biomarker for differential diagnosis of RCCs.

Acute tubular necrosis (ATN) is the most frequent cause of acute kidney injury, but there is no clinically available noninvasive tool to differentially diagnose ATN from other similar disorders, such as glomerulonephritis (GN). An *in vivo* mouse study showed that the conversion of hyperpolarized [$1,4\text{-}^{13}\text{C}_2$]fumarate to malate was significantly greater in the ATN cohort compared to the healthy or GN cohorts [264], indicating that hyperpolarized [$1,4\text{-}^{13}\text{C}_2$]fumarate could be a useful biomarker in providing differentiable diagnostic information on the causes of acute kidney injury.

Hyperpolarized [$1\text{-}^{13}\text{C}$]pyruvate has also been used to study metabolic changes in the renal system that follow the onset of diabetes. An *in vivo* rat study using a streptozotocin diabetes model showed a 149% increase in the lactate to pyruvate ratio of diabetic rats compared to control rats [265]. Another *in vivo* study demonstrated that reduced oxygen availability lead to a 23% and 34% increase in lactate and alanine labeling, respectively, in diabetic rats, whereas no such difference was observed in the control cohort. [266].

6.6. Lung

Hyperpolarized ^{13}C metabolic imaging of the lungs is quite challenging because of low tissue density, high susceptibility/ B_0 inhomogeneity due to air-tissue interfaces, motion, and the low metabolic rate of the lungs compared to solid organs [267]. On the other hand, the lung receives the full blood supply during each circulation and plays a key role in whole body homeostasis. As such, HP carbon MRI can be a valuable tool for evaluating lung metabolism and pathology.

HP ^{13}C MRI was first used in the porcine lung as a contrast agent to demonstrate feasibility as an angiography tool for evaluating pulmonary perfusion and imaging of pulmonary vasculature [76]. Metabolic imaging, however, is most easily performed using *ex vivo* perfused lungs when evaluating the suitability of specific hyperpolarized agents in different pulmonary disorders. HP [$1\text{-}^{13}\text{C}$]pyruvate spectroscopy of an ischemia-reperfusion model in perfused rat lungs showed a significant increase in HP lactate labeling after ischemia; the

lactate labeling was restored to baseline levels approximately 30–40 minutes after reperfusion [174]. A greater than four-fold increase in HP pyruvate-to-lactate ratio was also found during the inflammatory phase of a bleomycin induced lung injury model in perfused rat lungs, which was well correlated with the neutrophil count via histology (figure F) [124]. Another HP [1-¹³C]pyruvate study investigated the mechanism by which ascorbate prolongs the viability of isolated perfused lungs [164]. It was found that ascorbate enhanced viability via interaction with the electron transport chain, as opposed to its more common role as a general antioxidant. HP signal dynamics and the response to perfusate contents in the healthy lung was addressed using a three-compartment model to confirm the experimental finding [268] that HP lactate labeling is primarily determined by the rate at which NAD⁺ is reduced to NADH, and scales with the intracellular lactate pool size [167].

Current research is focused on moving towards *in vivo* HP ¹³C lung imaging. The first such *in vivo* study in rats demonstrated an increase in HP lactate labeling in a radiation induced lung injury (RILI) model, compared to controls [269]. The results were consistent with lung inflammation, as measured from bronchioalveolar lavage.

6.7. Muscle

Several findings related to hyperpolarized metabolism in skeletal muscle have been ancillary to the main study focus on other organs (e.g., liver or kidney) or in comparison to those organs. In *in vivo* rat studies of the peritoneum, HP [1-¹³C]pyruvate HP alanine labeling was comparable to—or slightly greater than—HP lactate labeling in muscles [122,259]. This metabolic profile is similar to the metabolic profile of the liver, but differs from that of the kidney, in which HP lactate labeling dominates. On the other hand, *in vivo* spectroscopy of porcine hind legs showed that HP lactate signal was about 2.5-fold greater than the HP alanine signal [122]. Given the lack of conclusive studies, it is reasonable to suggest the fate of the injected HP pyruvate may be highly dependent on the particular skeletal muscle being imaged, feeding status, or animal species.

A study specifically focused on skeletal muscle metabolism demonstrated that kinetic parameters derived from multiple bolus injections of HP [1-¹³C]pyruvate timed with a frequency- and amplitude-dependent stimuli-triggered activation of murine hind-limb muscles can be used for functional experiments of muscular activation (figure G7) [270]. Another study in rats demonstrated that the conversion of [1-¹³C]acetate to [1-¹³C]acetylcarnitine could be used as a biomarker for metabolic activity in skeletal muscle studies [184]. Although this work only focused on modeling enzymatic activity of acetyl-CoA synthetase (ACS) in healthy rats, the technique could be useful in a variety of pathologies that affect ACS activity. A third study examined muscle metabolism of hind legs in rats during fed and fasted states using [1-¹³C]lactate [271]. The advantage of using HP lactate instead of HP pyruvate as a tracer is that the delivered concentration of lactate is in the endogenous range and can be used to study the pool sizes of pyruvate and alanine, which seem to increase and decrease, respectively, in the fasted state.

6.8. Breast

Breast cancer is the most common female cancer in the United States. It's also the second leading cause of cancer death in women [272]. Breast cancer is normally considered curable if found and treated at the early stages. In fact, the mortality has dropped by one-third as compared to 1990 [273] due to increased screening and improved targeted treatments. Hyperpolarized ^{13}C as an emerging imaging technique can be used to detect malignancy, tumor progression, and response to therapy.

Several *in vitro* HP carbon MRI studies have been performed to demonstrate the feasibility of detecting metabolism in live cancer cells. Modulation of the environment to hypoxic conditions and lower perfusion rates increased the observed rate of pyruvate to lactate conversion by up-regulation of the monocarboxylate transporter 1 (MCT1). Conversely, a MCT1 inhibitor suppressed the conversion rate [123]. Treatment with mitogen-activated protein kinase (MEK) U0126 in both prostate and breast cancer cells showed significant decrease in the conversion of pyruvate to lactate in MCF-7 breast cancer cells, but not PC3 prostate cancer cells [274].

Breast tumor growth has also been monitored using HP MRI in *in vivo* mouse models. Models derived from several cell types, and their respective HP $[1-^{13}\text{C}]$ pyruvate to HP lactate kinetic parameters, have been compared to better understand the relationship between aggressiveness and the observed lactate labeling rate [24]. In another HP $[1-^{13}\text{C}]$ pyruvate study it was shown that the apparent kinetic rate of LDH correlated well with tumor growth. Treatment with the anti-estrogen tamoxifen delayed tumor progression, which correlated with the histological and *in vitro* LDH activity analysis [109].

7. Conclusion

The research community has made considerable advancements over fifteen years of investigating hyperpolarized injectable agents for use in medical imaging. Tangible progress has been made towards the goals of developing affordable HP MRI biomedical applications and robust use of this technology in human studies [275,276].

The technical improvements achieved by HP liquid MR research are clearly visible in labs around the world. In much the same manner as hyperpolarized gas technology a decade earlier, molecular imaging using DNP-hyperpolarized compounds has progressed from reliance on homemade equipment to the use of commercial devices suitable for *in vitro* and small animal work and finally to equipment that can safely and efficiently produce large samples appropriate for human trials. PHIP has also undergone a technical renaissance, as the field has been reenergized by recent demonstrations of spin-order transfer in transient catalytic complexes and chemical modification of the hyperpolarized compound.

Just as important, novel imaging methods have been introduced that improve acquisition speed, efficient use of the hyperpolarized state, metabolite selectivity, spatial and temporal resolution, and immunity to motion artifact. Alongside this work has been a vigorous effort to extract the quantitative information (e.g., rate uptake and metabolic flux) necessary to attain a fundamental understanding of metabolic signals by combining the imaging results

with kinetic modeling. Some of the most promising work highlighted in the organ-specific discussion relies on these image acquisition and analysis procedural improvements. For example, the primary focus in HP MR heart studies has been quantification of PDH flux and its modification with physiological stress or pharmaceutical intervention. Similar efforts have been pursued in isolated, perfused organs, for which the arterial input function is known, and in brain and liver, for which it is measured. These studies have already yielded unique quantitative metabolic flux and exchange data as well as detection of low-concentration intermediates, and can naturally be extended to quantitative perfusion and quantitative flux studies in any organ and in tumors.

The central challenge moving forward will be the translation of sequences and modeling techniques suitable for quantification into the clinic, with the aim of better understanding the relationship between these new measurements and human disease. Human studies have begun after the successful phase I trial that demonstrated the initial safety of the HP technique at the University of California, San Francisco in 2013. As of this writing, these translational efforts are being pursued at two sites worldwide. That number will grow dramatically in the immediate future as the solutions to technical and regulatory challenges are disseminated.

References

1. Joint SNM/RSNA Molecular Imaging Summit Statement. *J Nucl Med.* 2005; 46:11N–42N.
2. Hoh CK. Clinical use of FDG PET. *Nucl Med Biol.* 2007; 34:737–742. DOI: 10.1016/j.nucmedbio.2007.07.001 [PubMed: 17921026]
3. Popovtzer R, Agrawal A, Kotov NA, Popovtzer A, Balter J, Carey TE, Kopelman R. Targeted Gold Nanoparticles Enable Molecular CT Imaging of Cancer. *Nano Lett.* 2008; 8:4593–4596. DOI: 10.1021/nl8029114 [PubMed: 19367807]
4. James ML, Gambhir SS. A molecular imaging primer: modalities, imaging agents, and applications. *Physiol Rev.* 2012; 92:897–965. DOI: 10.1152/physrev.00049.2010 [PubMed: 22535898]
5. Hoult DI, Busby SJ, Gadian DG, Radda GK, Richards RE, Seeley PJ. Observation of tissue metabolites using ³¹P nuclear magnetic resonance. *Nature.* 1974; 252:285–287. [PubMed: 4431445]
6. Weiss RG, Chacko VP, Gerstenblith G. Fatty acid regulation of glucose metabolism in the intact beating rat heart assessed by carbon-13 NMR spectroscopy: the critical role of pyruvate dehydrogenase. *J Mol Cell Cardiol.* 1989; 21:469–478. [PubMed: 2528640]
7. Reddy R, Insko EK, Noyszewski EA, Dandora R, Kneeland JB, Leigh JS. Sodium MRI of human articular cartilage in vivo. *Magn Reson Med.* 1998; 39:697–701. [PubMed: 9581599]
8. Rudd JHF, Warburton EA, Fryer TD, Jones HA, Clark JC, Antoun N, Johnström P, Davenport AP, Kirkpatrick PJ, Arch BN, Pickard JD, Weissberg PL. Imaging atherosclerotic plaque inflammation with [¹⁸F]-fluorodeoxyglucose positron emission tomography. *Circulation.* 2002; 105:2708–2711. [PubMed: 12057982]
9. Lucignani G, Paolini G, Landoni C, Zuccari M, Paganelli G, Galli L, Di Credico G, Vanoli G, Rossetti C, Mariani MA. Presurgical identification of hibernating myocardium by combined use of technetium-99m hexakis 2-methoxyisobutylisonitrile single photon emission tomography and fluorine-18 fluoro-2-deoxy-D-glucose positron emission tomography in patients with coronary artery disease. *Eur J Nucl Med.* 1992; 19:874–881. [PubMed: 1451704]
10. Trani D, Yaromina A, Dubois L, Granzier M, Peeters SGJA, Biemans R, Nalbantov G, Lieuwes N, Reniers B, Troost EEC, Verhaegen F, Lambin P. Preclinical Assessment of Efficacy of Radiation Dose Painting Based on Intratumoral FDG-PET Uptake. *Clin Cancer Res.* 2015; 21:5511–5518. DOI: 10.1158/1078-0432.CCR-15-0290 [PubMed: 26276892]

11. Miyamoto M, Kuroda S, Zhao S, Magota K, Shichinohe H, Houkin K, Kuge Y, Tamaki N. Bone Marrow Stromal Cell Transplantation Enhances Recovery of Local Glucose Metabolism After Cerebral Infarction in Rats: A Serial 18F-FDG PET Study. *J Nucl Med.* 2013; 54:145–150. DOI: 10.2967/jnumed.112.109017 [PubMed: 23204494]
12. Khalil MM, Tremoleda JL, Bayomy TB, Gsell W, Khalil MM, Tremoleda JL, Bayomy TB, Gsell W. Molecular SPECT Imaging: An Overview, Molecular SPECT Imaging: An Overview. *Int J Mol Imaging* 2011. 2011; 2011:e796025.doi: 10.1155/2011/796025
13. Franc BL, Acton PD, Mari C, Hasegawa BH. Small-Animal SPECT and SPECT/CT: Important Tools for Preclinical Investigation. *J Nucl Med.* 2008; 49:1651–1663. DOI: 10.2967/jnumed.108.055442 [PubMed: 18794275]
14. Gambhir SS. Molecular imaging of cancer with positron emission tomography. *Nat Rev Cancer.* 2002; 2:683–693. DOI: 10.1038/nrc882 [PubMed: 12209157]
15. Fletcher JW, Djulbegovic B, Soares HP, Siegel BA, Lowe VJ, Lyman GH, Coleman RE, Wahl R, Paschold JC, Avril N, Einhorn LH, Suh WW, Samson D, Delbeke D, Gorman M, Shields AF. Recommendations on the use of 18F-FDG PET in oncology. *J Nucl Med Off Publ Soc Nucl Med.* 2008; 49:480–508. DOI: 10.2967/jnumed.107.047787
16. Kubota K. From tumor biology to clinical Pet: a review of positron emission tomography (PET) in oncology. *Ann Nucl Med.* 2001; 15:471–486. [PubMed: 11831394]
17. Lopci E, Nanni C, Castellucci P, Montini GC, Allegri V, Rubello D, Chierichetti F, Ambrosini V, Fanti S. Imaging with non-FDG PET tracers: outlook for current clinical applications. *Insights Imaging.* 2010; 1:373–385. DOI: 10.1007/s13244-010-0040-9
18. Belhocine T, Steinmetz N, Hustinx R, Bartsch P, Jerusalem G, Seidel L, Rigo P, Green A. Increased uptake of the apoptosis-imaging agent (99m)Tc recombinant human Annexin V in human tumors after one course of chemotherapy as a predictor of tumor response and patient prognosis. *Clin Cancer Res Off J Am Assoc Cancer Res.* 2002; 8:2766–2774.
19. Matthews PM, Rabiner EA, Passchier J, Gunn RN. Positron emission tomography molecular imaging for drug development. *Br J Clin Pharmacol.* 2012; 73:175–186. DOI: 10.1111/j.1365-2125.2011.04085.x [PubMed: 21838787]
20. Huang WS, Lin SZ, Lin JC, Wey SP, Ting G, Liu RS. Evaluation of early-stage Parkinson's disease with 99mTc-TRODAT-1 imaging. *J Nucl Med Off Publ Soc Nucl Med.* 2001; 42:1303–1308.
21. Beyer T, Townsend DW, Brun T, Kinahan PE, Charron M, Roddy R, Jerin J, Young J, Byars L, Nutt R. A Combined PET/CT Scanner for Clinical Oncology. *J Nucl Med.* 2000; 41:1369–1379. [PubMed: 10945530]
22. Pichler BJ, Judenhofer MS, Pfannenberger C. Multimodal imaging approaches: PET/CT and PET/MRI. *Handb Exp Pharmacol.* 2008; :109–132. DOI: 10.1007/978-3-540-72718-7_6 [PubMed: 18626801]
23. Tyler DJ, Schroeder MA, Cochlin LE, Clarke K, Radda GK. Application of Hyperpolarized Magnetic Resonance in the Study of Cardiac Metabolism. *Appl Magn Reson.* 2008; 34:523–531. DOI: 10.1007/s00723-008-0115-7
24. Daniels CJ, McLean MA, Schulte RF, Robb FJ, Gill AB, McGlashan N, Graves MJ, Schwaiger M, Lomas DJ, Brindle KM, Gallagher FA. A comparison of quantitative methods for clinical imaging with hyperpolarized 13C-pyruvate. *NMR Biomed.* 2016; 29:387–399. DOI: 10.1002/nbm.3468 [PubMed: 27414749]
25. Weissleder R, Ntziachristos V. Shedding light onto live molecular targets. *Nat Med.* 2003; 9:123–128. DOI: 10.1038/nm0103-123 [PubMed: 12514725]
26. van Dam GM, Themelis G, Crane LMA, Harlaar NJ, Pleijhuis RG, Kelder W, Sarantopoulos A, de Jong JS, Arts HJG, van der Zee AGJ, Bart J, Low PS, Ntziachristos V. Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor- α targeting: first in-human results. *Nat Med.* 2011; 17:1315–1319. DOI: 10.1038/nm.2472 [PubMed: 21926976]
27. [accessed May 20, 2016] Long-Residence-Time Nano-Scale Liposomal Iohexol for X-ray-Based Blood Pool Imaging - Academic Radiology. n.d. [http://www.academicradiology.org/article/S1076-6332\(03\)80055-7/abstract](http://www.academicradiology.org/article/S1076-6332(03)80055-7/abstract)

28. Schmiedl UP, Krause W, Leike J, Sachse A. CT blood pool enhancement in primates with iopromide-carrying liposomes containing soy phosphatidyl glycerol. *Acad Radiol.* 1999; 6:164–169. DOI: 10.1016/S1076-6332(99)80402-4 [PubMed: 10898035]
29. Hyafil F, Cornily JC, Feig JE, Gordon R, Vucic E, Amirbekian V, Fisher EA, Fuster V, Feldman LJ, Fayad ZA. Noninvasive detection of macrophages using a nanoparticulate contrast agent for computed tomography. *Nat Med.* 2007; 13:636–641. DOI: 10.1038/nm1571 [PubMed: 17417649]
30. Samei E, Saunders RS, Badea CT, Ghaghada KB, Hedlund LW, Qi Y, Yuan H, Bentley RC, Mukundan S. Micro-CT imaging of breast tumors in rodents using a liposomal, nanoparticle contrast agent. *Int J Nanomedicine.* 2009; 4:277–282. [PubMed: 20011244]
31. Karathanasis E, Chan L, Karumbaiah L, McNeeley K, D’Orsi CJ, Annappagada AV, Sechopoulos I, Bellamkonda RV. Tumor Vascular Permeability to a Nanoprobe Correlates to Tumor-Specific Expression Levels of Angiogenic Markers. *PLOS ONE.* 2009; 4:e5843.doi: 10.1371/journal.pone.0005843 [PubMed: 19513111]
32. Danila D, Partha R, Elrod DB, Lackey M, Casscells SW, Conyers JL. Antibody-labeled liposomes for CT imaging of atherosclerotic plaques: in vitro investigation of an anti-ICAM antibody-labeled liposome containing iohexol for molecular imaging of atherosclerotic plaques via computed tomography. *Tex Heart Inst J Tex Heart Inst St Lukes Episcop Hosp Tex Child Hosp.* 2009; 36:393–403.
33. Lusic H, Grinstaff MW. X-ray-Computed Tomography Contrast Agents. *Chem Rev.* 2013; 113:1641–1666. DOI: 10.1021/cr200358s [PubMed: 23210836]
34. Cai QY, Kim SH, Choi KS, Kim SY, Byun SJ, Kim KW, Park SH, Juhng SK, Yoon KH. Colloidal gold nanoparticles as a blood-pool contrast agent for X-ray computed tomography in mice. *Invest Radiol.* 2007; 42:797–806. DOI: 10.1097/RLI.0b013e31811ecdcd [PubMed: 18007151]
35. Wang H, Zheng L, Peng C, Guo R, Shen M, Shi X, Zhang G. Computed tomography imaging of cancer cells using acetylated dendrimer-entrapped gold nanoparticles. *Biomaterials.* 2011; 32:2979–2988. DOI: 10.1016/j.biomaterials.2011.01.001 [PubMed: 21277019]
36. Zhang Z, Ross RD, Roeder RK. Preparation of functionalized gold nanoparticles as a targeted X-ray contrast agent for damaged bone tissue. *Nanoscale.* 2010; 2:582–586. DOI: 10.1039/B9NR00317G [PubMed: 20644762]
37. Chanda N, Kattumuri V, Shukla R, Zambre A, Katti K, Upendran A, Kulkarni RR, Kan P, Fent GM, Casteel SW, Smith CJ, Boote E, Robertson JD, Cutler C, Lever JR, Katti KV, Kannan R. Bombesin functionalized gold nanoparticles show in vitro and in vivo cancer receptor specificity. *Proc Natl Acad Sci.* 2010; 107:8760–8765. DOI: 10.1073/pnas.1002143107 [PubMed: 20410458]
38. Cormode DP, Naha PC, Fayad ZA. Nanoparticle Contrast Agents for Computed Tomography: A Focus on Micelles. *Contrast Media Mol Imaging.* 2014; 9:37–52. DOI: 10.1002/cmmi.1551 [PubMed: 24470293]
39. Song KH, Kim C, Cogley CM, Xia Y, Wang LV. Near-Infrared Gold Nanocages as a New Class of Tracers for Photoacoustic Sentinel Lymph Node Mapping on a Rat Model. *Nano Lett.* 2009; 9:183–188. DOI: 10.1021/nl802746w [PubMed: 19072058]
40. Agarwal A, Huang SW, O’Donnell M, Day KC, Day M, Kotov N, Ashkenazi S. Targeted gold nanorod contrast agent for prostate cancer detection by photoacoustic imaging. *J Appl Phys.* 2007; 102:064701.doi: 10.1063/1.2777127
41. Happer W. Optical Pumping. *Rev Mod Phys.* 1972; 44:169–249. DOI: 10.1103/RevModPhys.44.169
42. Walker TG, Happer W. Spin-exchange optical pumping of noble-gas nuclei. *Rev Mod Phys.* 1997; 69:629.
43. Schmidt AB, Andrews DL, Rohrbach A, Gohn-Kreuz C, Shatokhin VN, Kiselev VG, Hennig J, von Elverfeldt D, Hövener JB. Do twisted laser beams evoke nuclear hyperpolarization? *J Magn Reson San Diego Calif* 1997. 2016; 268:58–67. DOI: 10.1016/j.jmr.2016.04.015
44. Overhauser AW. Polarization of nuclei in metals. *Phys Rev.* 1953; 92:411.
45. Carver TR, Slichter CP. Polarization of nuclear spins in metals. *Phys Rev.* 1953; 92:212.
46. Flori A, Liserani M, Bowen S, Ardenkjaer-Larsen JH, Menichetti L. Dissolution Dynamic Nuclear Polarization of Non-Self-Glassing Agents: Spectroscopy and Relaxation of Hyperpolarized

- [1-¹³C]Acetate. *J Phys Chem A*. 2015; 119:1885–1893. DOI: 10.1021/jp511972g [PubMed: 25686013]
47. Abragam A, Goldman M. Principles of dynamic nuclear polarisation. *Rep Prog Phys*. 1978; 41:395.doi: 10.1088/0034-4885/41/3/002
 48. Barnes AB, De Paëpe G, van der Wel PCA, Hu KN, Joo CG, Bajaj VS, Mak-Jurkauskas ML, Sirigiri JR, Herzfeld J, Temkin RJ, Griffin RG. High-Field Dynamic Nuclear Polarization for Solid and Solution Biological NMR. *Appl Magn Reson*. 2008; 34:237–263. DOI: 10.1007/s00723-008-0129-1 [PubMed: 19194532]
 49. Hovav Y, Feintuch A, Vega S. Theoretical aspects of dynamic nuclear polarization in the solid state – spin temperature and thermal mixing. *Phys Chem Chem Phys*. 2013; 15:188–203. DOI: 10.1039/C2CP42897K [PubMed: 23160533]
 50. Goldman M. Overview of Spin Temperature, Thermal Mixing and Dynamic Nuclear Polarization. *Appl Magn Reson*. 2008; 34:219–226. DOI: 10.1007/s00723-008-0114-8
 51. Osten HJ, Jameson CJ. Quadrupolar spin relaxation due to electric field gradients induced by vibrations and collisions. *Mol Phys*. 1986; 57:553–571. DOI: 10.1080/00268978600100411
 52. Karlsson M, Jensen PR, Duus JØ, Meier S, Lerche MH. Development of Dissolution DNP-MR Substrates for Metabolic Research. *Appl Magn Reson*. 2012; 43:223–236. DOI: 10.1007/s00723-012-0336-7
 53. Chattergoon N, Martínez-Santesteban F, Handler WB, Ardenkjær-Larsen JH, Scholl TJ. Field dependence of T1 for hyperpolarized [1-¹³C] pyruvate. *Contrast Media Mol Imaging*. 2013; 8:57–62. [PubMed: 23109393]
 54. Lumata L, Merritt ME, Kovacs Z. Influence of deuteration in the glassing matrix on ¹³C dynamic nuclear polarization. *Phys Chem Chem Phys*. 2013; 15:7032.doi: 10.1039/c3cp50750e [PubMed: 23552448]
 55. Cancer Imaging Program, Hyperpolarized Pyruvate (¹³C) Injection: Investigator's Brochure. 2016. http://imaging.cancer.gov/images/documents/c13_pyruvate_IB_PDF
 56. Kuzma NN, Pourfathi M, Kara H, Manasseh P, Ghosh RK, Ardenkjær-Larsen JH, Kadlecsek SJ, Rizi RR. Cluster formation restricts dynamic nuclear polarization of xenon in solid mixtures. *J Chem Phys*. 2012; 137:104508. [PubMed: 22979875]
 57. Muñoz-Gómez JL, Monteagudo E, Lloveras V, Parella T, Veciana J, Vidal-Gancedo J. A benzyl alcohol derivative of the BDPA radical for fast dissolution dynamic nuclear polarization NMR spectroscopy. *Org Biomol Chem*. 2015; 13:2689–2693. DOI: 10.1039/C4OB02356K [PubMed: 25586215]
 58. Haze O, Corzilius B, Smith AA, Griffin RG, Swager TM. Water-Soluble Narrow-Line Radicals for Dynamic Nuclear Polarization. *J Am Chem Soc*. 2012; 134:14287–14290. DOI: 10.1021/ja304918g [PubMed: 22917088]
 59. McCarney ER, Armstrong BD, Lingwood MD, Han S. Hyperpolarized water as an authentic magnetic resonance imaging contrast agent. *Proc Natl Acad Sci*. 2007; 104:1754–1759. [PubMed: 17264210]
 60. Ardenkjær-Larsen, JH., Laustsen, C., Pullinger, B., Kadlecsek, S., Emami, K., Rizi, R. [accessed June 9, 2016] Hyperpolarized Water for Interventional Angiography; *Proc Int Soc Mag Reson Med*. 2011. p. 3534<http://cds.ismrm.org/protected/11MProceedings/files/3534.pdf>
 61. Lingwood MD, Siaw TA, Sailasuta N, Abulseoud OA, Chan HR, Ross BD, Bhattacharya P, Han S. Hyperpolarized water as an MR imaging contrast agent: feasibility of in vivo imaging in a rat model. *Radiology*. 2012; 265:418–425. [PubMed: 22996746]
 62. Song C, Hu KN, Joo CG, Swager TM, Griffin RG. TOTAPOL: A Biradical Polarizing Agent for Dynamic Nuclear Polarization Experiments in Aqueous Media. *J Am Chem Soc*. 2006; 128:11385–11390. DOI: 10.1021/ja061284b [PubMed: 16939261]
 63. Matsuki Y, Maly T, Ouari O, Karoui H, Le Moigne F, Rizzato E, Lyubenova S, Herzfeld J, Prisner T, Tordo P, Griffin RG. Dynamic Nuclear Polarization with a Rigid Biradical. *Angew Chem Int Ed*. 2009; 48:4996–5000. DOI: 10.1002/anie.200805940
 64. Hu KN, Yu H, Swager TM, Griffin RG. Dynamic Nuclear Polarization with Biradicals. *J Am Chem Soc*. 2004; 126:10844–10845. DOI: 10.1021/ja039749a [PubMed: 15339160]

65. Eichhorn TR, Takado Y, Salameh N, Capozzi A, Cheng T, Hyacinthe JN, Mishkovsky M, Roussel C, Comment A. Hyperpolarization without persistent radicals for in vivo real-time metabolic imaging. *Proc Natl Acad Sci*. 2013; 110:18064–18069. DOI: 10.1073/pnas.1314928110 [PubMed: 24145405]
66. Ardenkjaer-Larsen JH, Macholl S, Jóhannesson H. Dynamic Nuclear Polarization with Trityls at 1.2 K. *Appl Magn Reson*. 2008; 34:509–522. DOI: 10.1007/s00723-008-0134-4
67. Hurd RE, Yen YF, Chen A, Ardenkjaer-Larsen JH. Hyperpolarized ^{13}C metabolic imaging using dissolution dynamic nuclear polarization. *J Magn Reson Imaging*. 2012; 36:1314–1328. DOI: 10.1002/jmri.23753 [PubMed: 23165733]
68. Walker SA, Edwards DT, Siaw TA, Armstrong BD, Han S. Temperature dependence of high field ^{13}C dynamic nuclear polarization processes with trityl radicals below 35 Kelvin. *Phys Chem Chem Phys*. 2013; 15:15106. doi: 10.1039/c3cp51628h [PubMed: 23925724]
69. Jannin S, Comment A, Kurdziesau F, Konter JA, Hautle P, van den Brandt B, van der Klink JJ. A 140 GHz prepolarizer for dissolution dynamic nuclear polarization. *J Chem Phys*. 2008; 128:241102. doi: 10.1063/1.2951994 [PubMed: 18601309]
70. Shang H, Skloss T, von Morze C, Carvajal L, Van Criekeing M, Milshteyn E, Larson PEZ, Hurd RE, Vigneron DB. Handheld electromagnet carrier for transfer of hyperpolarized carbon-13 samples. *Magn Reson Med*. 2015; :n/a–n/a. DOI: 10.1002/mrm.25657
71. Bargon J, Fischer H, Johnsen U. Nuclear magnetic resonance emission lines during fast radical reactions. I. Recording methods and examples. *Z Naturforsch A*. 1967; 22:1551–1555.
72. Ward HR, Lawler RG. Nuclear magnetic resonance emission and enhanced absorption in rapid organometallic reactions. *J Am Chem Soc*. 1967; 89:5518–5519.
73. Bowers CR, Weitekamp DP. Transformation of symmetrization order to nuclear-spin magnetization by chemical reaction and nuclear magnetic resonance. *Phys Rev Lett*. 1986; 57:2645. [PubMed: 10033824]
74. Natterer J, Bargon J. Parahydrogen induced polarization. *Prog Nucl Magn Reson Spectrosc*. 1997; 31:293–315. DOI: 10.1016/S0079-6565(97)00007-1
75. Bhattacharya P, Chekmenev EY, Reynolds WF, Wagner S, Zacharias N, Chan HR, Bünger R, Ross BD. Parahydrogen-induced polarization (PHIP) hyperpolarized MR receptor imaging in vivo: a pilot study of ^{13}C imaging of atheroma in mice: Towards Imaging Plaque In Vivo using ^{13}C Hyperpolarization. *NMR Biomed*. 2011; 24:1023–1028. DOI: 10.1002/nbm.1717 [PubMed: 21538638]
76. Ishii M, Emami K, Kadlecsek S, Petersson JS, Golman K, Vahdat V, Yu J, Cadman RV, MacDuffie-Woodburn J, Stephen M, Lipson DA, Rizi RR. Hyperpolarized ^{13}C MRI of the pulmonary vasculature and parenchyma. *Magn Reson Med*. 2007; 57:459–463. DOI: 10.1002/mrm.21168 [PubMed: 17326170]
77. Zacharias NM, Chan HR, Sailasuta N, Ross BD, Bhattacharya P. Real-Time Molecular Imaging of Tricarboxylic Acid Cycle Metabolism in Vivo by Hyperpolarized 1- ^{13}C Diethyl Succinate. *J Am Chem Soc*. 2012; 134:934–943. DOI: 10.1021/ja2040865 [PubMed: 22146049]
78. Coffey AM, Shchepin RV, Truong ML, Wilkens K, Pham W, Chekmenev EY. Open-Source Automated Parahydrogen Hyperpolarizer for Molecular Imaging Using (^{13}C) Metabolic Contrast Agents. *Anal Chem*. 2016; 88:8279–8288. DOI: 10.1021/acs.analchem.6b02130 [PubMed: 27478927]
79. Juarez AM, Cubric D, King GC. A compact catalytic converter for the production of parahydrogen. *Meas Sci Technol*. 2002; 13:N52.
80. Weitzel D, Loebenstein W, Draper J, Park O. Ortho-Para Catalysis in Liquid-Hydrogen Production. *J Res Natl Bur Stand*. 1958; 60:221.
81. Morris GA, Freeman R. Enhancement of nuclear magnetic resonance signals by polarization transfer. *J Am Chem Soc*. 1979; 101:760–762.
82. Kadlecsek S, Emami K, Ishii M, Rizi R. Optimal transfer of spin-order between a singlet nuclear pair and a heteronucleus. *J Magn Reson*. 2010; 205:9–13. DOI: 10.1016/j.jmr.2010.03.004 [PubMed: 20427216]

83. Barkemeyer J, Bargon J, Sengstschmid H, Freeman R. Heteronuclear Polarization Transfer Using Selective Pulses during Hydrogenation with Parahydrogen. *J Magn Reson.* 1996; 120:129–132. DOI: 10.1006/jmra.1996.0109
84. Haake M, Natterer J, Bargon J. Efficient NMR Pulse Sequences to Transfer the Parahydrogen-Induced Polarization to Hetero Nuclei. *J Am Chem Soc.* 1996; 118:8688–8691. DOI: 10.1021/ja960067f
85. Goldman M, Jóhannesson H. Conversion of a proton pair para order into ¹³C polarization by rf irradiation, for use in MRI. *Comptes Rendus Phys.* 2005; 6:575–581. DOI: 10.1016/j.crhy.2005.03.002
86. Golman K, Axelsson O, Jóhannesson H, Månsson S, Olofsson C, Petersson JS. Parahydrogen-induced polarization in imaging: Subsecond ¹³C angiography. *Magn Reson Med.* 2001; 46:1–5. [PubMed: 11443703]
87. Jóhannesson H, Axelsson O, Karlsson M. Transfer of para-hydrogen spin order into polarization by diabatic field cycling. *Comptes Rendus Phys.* 2004; 5:315–324. DOI: 10.1016/j.crhy.2004.02.001
88. Kadlecsek S, Vahdat V, Nakayama T, Ng D, Emami K, Rizi R. A simple and low-cost device for generating hyperpolarized contrast agents using parahydrogen: LOW-COST IMPLEMENTATION OF PARAHYDROGEN-INDUCED POLARIZATION. *NMR Biomed.* 2011; 24:933–942. DOI: 10.1002/nbm.1757 [PubMed: 21845739]
89. Shchepin RV, Coffey AM, Waddell KW, Chekmenev EY. Parahydrogen Induced Polarization of ¹⁻¹³C-Phospholactate-*d*₂ for Biomedical Imaging with >30,000,000-fold NMR Signal Enhancement in Water. *Anal Chem.* 2014; 86:5601–5605. DOI: 10.1021/ac500952z [PubMed: 24738968]
90. Reineri F, Boi T, Aime S. ParaHydrogen Induced Polarization of ¹³C carboxylate resonance in acetate and pyruvate. *Nat Commun.* 2015; 6:5858.doi: 10.1038/ncomms6858 [PubMed: 25556844]
91. Shchepin RV, Barskiy DA, Coffey AM, Manzanera Esteve IV, Chekmenev EY. Efficient Synthesis of Molecular Precursors for Para-Hydrogen-Induced Polarization of Ethyl Acetate-1-¹³C and Beyond. *Angew Chem Int Ed.* 2016; 55:6071–6074. DOI: 10.1002/anie.201600521
92. Cavallari E, Carrera C, Boi T, Aime S, Reineri F. Effects of Magnetic Field Cycle on the Polarization Transfer from Parahydrogen to Heteronuclei through Long-Range J-Couplings. *J Phys Chem B.* 2015; 119:10035–10041. DOI: 10.1021/acs.jpcc.5b06222 [PubMed: 26161454]
93. Gridnev ID, Higashi N, Asakura K, Imamoto T. Mechanism of Asymmetric Hydrogenation Catalyzed by a Rhodium Complex of (*S,S*)-1,2-Bis(*tert*-butylmethylphosphino)ethane. Dihydride Mechanism of Asymmetric Hydrogenation. *J Am Chem Soc.* 2000; 122:7183–7194. DOI: 10.1021/ja000813n
94. Burgess JM, Molloy NA. Gas absorption in the plunging liquid jet reactor. *Chem Eng Sci.* 1973; 28:183–190.
95. Hövener J-B, Chekmenev EY, Harris KC, Perman WH, Robertson LW, Ross BD, Bhattacharya P. PASADENA hyperpolarization of ¹³C biomolecules: equipment design and installation. *Magn Reson Mater Phys Biol Med.* 2009; 22:111–121. DOI: 10.1007/s10334-008-0155-x
96. Goldman M, Jóhannesson H, Axelsson O, Karlsson M. Design and implementation of ¹³C hyper polarization from para-hydrogen, for new MRI contrast agents. *Comptes Rendus Chim.* 2006; 9:357–363. DOI: 10.1016/j.crci.2005.05.010
97. Glöggler S, Grunfeld AM, Ertas YN, McCormick J, Wagner S, Schleker PPM, Bouchard L-S. A Nanoparticle Catalyst for Heterogeneous Phase Para-Hydrogen-Induced Polarization in Water. *Angew Chem Int Ed.* 2015; 54:2452–2456. DOI: 10.1002/anie.201409027
98. Bhattacharya P, Chekmenev EY, Perman WH, Harris KC, Lin AP, Norton VA, Tan CT, Ross BD, Weitekamp DP. Towards hyperpolarized ¹³C-succinate imaging of brain cancer. *J Magn Reson.* 2007; 186:150–155. DOI: 10.1016/j.jmr.2007.01.017 [PubMed: 17303454]
99. Ross BD, Bhattacharya P, Wagner S, Tran T, Sailasuta N. Hyperpolarized MR Imaging: Neurologic Applications of Hyperpolarized Metabolism. *Am J Neuroradiol.* 2010; 31:24–33. DOI: 10.3174/ajnr.A1790 [PubMed: 19875468]
100. Adams RW, Aguilar JA, Atkinson KD, Cowley MJ, Elliott PIP, Duckett SB, Green GGR, Khazal IG, Lopez-Serrano J, Williamson DC. Reversible Interactions with para-Hydrogen Enhance

NMR Sensitivity by Polarization Transfer. *Science*. 2009; 323:1708–1711. DOI: 10.1126/science.1168877 [PubMed: 19325111]

101. Ardenkjær-Larsen JH, Fridlund B, Gram A, Hansson G, Hansson L, Lerche MH, Servin R, Thaning M, Golman K. Increase in signal-to-noise ratio of > 10,000 times in liquid-state NMR. *Proc Natl Acad Sci*. 2003; 100:10158–10163. DOI: 10.1073/pnas.1733835100 [PubMed: 12930897]
102. Comment A. Dissolution DNP for in vivo preclinical studies. *J Magn Reson*. 2016; 264:39–48. DOI: 10.1016/j.jmr.2015.12.027 [PubMed: 26920829]
103. Nelson SJ, Kurhanewicz J, Vigneron DB, Larson PEZ, Harzstark AL, Ferrone M, van Criekinge M, Chang JW, Bok R, Park I, Reed G, Carvajal L, Small EJ, Munster P, Weinberg VK, Ardenkjær-Larsen JH, Chen AP, Hurd RE, Odegardstuen L-I, Robb FJ, Tropp J, Murray JA. Metabolic Imaging of Patients with Prostate Cancer Using Hyperpolarized [1-13C]Pyruvate. *Sci Transl Med*. 2013; 5:198ra108–198ra108. DOI: 10.1126/scitranslmed.3006070
104. Laustsen C, Hansen ESS, Kjaergaard U, Bertelsen LB, Ringgaard S, Stødkilde-Jørgensen H. Acute porcine renal metabolic effect of endogastric soft drink administration assessed with hyperpolarized [1-13C]pyruvate: Oral Sucrose Load Assessed with MRS in Porcine Kidney. *Magn Reson Med*. 2015; 74:558–563. DOI: 10.1002/mrm.25692 [PubMed: 26014387]
105. Giovannetti G, Frijia F, Menichetti L, Milanesi M, Ardenkjær-Larsen JH, De Marchi D, Hartwig V, Positano V, Landini L, Lombardi M, Santarelli MF. Hyperpolarized [sup 13C] MRS surface coil: Design and signal-to-noise ratio estimation. *Med Phys*. 2010; 37:5361.doi: 10.1118/1.3491437 [PubMed: 21089771]
106. Chen AP, Hurd RE, Schroeder MA, Lau AZ, Gu Y, Lam WW, Barry J, Tropp J, Cunningham CH. Simultaneous investigation of cardiac pyruvate dehydrogenase flux, Krebs cycle metabolism and pH, using hyperpolarized [1,2-13C2]pyruvate in vivo: HYPERPOLARIZED [1,2-13C2]PYRUVATE IN HEART. *NMR Biomed*. 2012; 25:305–311. DOI: 10.1002/nbm.1749 [PubMed: 21774012]
107. Park JM, Josan S, Jang T, Merchant M, Watkins R, Hurd RE, Recht LD, Mayer D, Spielman DM. Volumetric spiral chemical shift imaging of hyperpolarized [2-13C]pyruvate in a rat c6 glioma model. *Magn Reson Med*. 2015; :n/a–n/a. DOI: 10.1002/mrm.25766
108. Day SE, Kettunen MI, Gallagher FA, Hu D-E, Lerche M, Wolber J, Golman K, Ardenkjær-Larsen JH, Brindle KM. Detecting tumor response to treatment using hyperpolarized 13C magnetic resonance imaging and spectroscopy. *Nat Med*. 2007; 13:1382–1387. DOI: 10.1038/nm1650 [PubMed: 17965722]
109. Asghar Butt S, Sogaard LV, Ardenkjær-Larsen JH, Lauritzen MH, Engelholm LH, Paulson OB, Mirza O, Holck S, Magnusson P, Åkeson P. Monitoring mammary tumor progression and effect of tamoxifen treatment in MMTV-PyMT using MRI and magnetic resonance spectroscopy with hyperpolarized [1-13C]pyruvate. *Magn Reson Med*. 2015; 73:51–58. DOI: 10.1002/mrm.25095 [PubMed: 24435823]
110. Dominguez-Viqueira W, Geraghty BJ, Lau JYC, Robb FJ, Chen AP, Cunningham CH. Intensity correction for multichannel hyperpolarized ¹³C imaging of the heart: Intensity Correction for Multichannel ¹³C. *Magn Reson Med*. 2016; 75:859–865. DOI: 10.1002/mrm.26042 [PubMed: 26619820]
111. Sack M, Wetherling F, Sartorius A, Ende G, Weber-Fahr W. Signal-to-noise ratio of a mouse brain ¹³C CryoProbe™ system in comparison with room temperature coils: spectroscopic phantom and *in vivo* results: SNR OF A ¹³C CRYOPROBE™ IN COMPARISON WITH ROOM TEMPERATURE COILS. *NMR Biomed*. 2014; 27:709–715. DOI: 10.1002/nbm.3110 [PubMed: 24692120]
112. Kovacs H, Moskau D, Spraul M. Cryogenically cooled probes—a leap in NMR technology. *Prog Nucl Magn Reson Spectrosc*. 2005; 46:131–155. DOI: 10.1016/j.pnmrs.2005.03.001
113. Yen Y-F, Kohler SJ, Chen AP, Tropp J, Bok R, Wolber J, Albers MJ, Gram KA, Zierhut ML, Park I, Zhang V, Hu S, Nelson SJ, Vigneron DB, Kurhanewicz J, Dirven HAA, Hurd RE. Imaging considerations for in vivo ¹³C metabolic mapping using hyperpolarized ¹³C-pyruvate. *Magn Reson Med*. 2009; 62:1–10. DOI: 10.1002/mrm.21987 [PubMed: 19319902]

114. Lau AZ, Chen AP, Ghugre NR, Ramanan V, Lam WW, Connelly KA, Wright GA, Cunningham CH. Rapid multislice imaging of hyperpolarized ^{13}C pyruvate and bicarbonate in the heart. *Magn Reson Med*. 2010; 64:1323–1331. DOI: 10.1002/mrm.22525 [PubMed: 20574989]
115. Lau AZ, Miller JJ, Robson MD, Tyler DJ. Simultaneous assessment of cardiac metabolism and perfusion using copolarized $[1-^{13}\text{C}]$ pyruvate and ^{13}C -urea: Simultaneous Hyperpolarized Cardiac Perfusion and Metabolic Imaging. *Magn Reson Med*. 2016; :n/a–n/a. DOI: 10.1002/mrm.26106
116. Gordon JW, Niles DJ, Adamson EB, Johnson KM, Fain SB. Application of flow sensitive gradients for improved measures of metabolism using hyperpolarized ^{13}C MRI. *Magn Reson Med*. 2015; :n/a–n/a. DOI: 10.1002/mrm.25584
117. Svensson J, Månsson S, Johansson E, Petersson JS, Olsson LE. Hyperpolarized ^{13}C MR angiography using trueFISP: Hyperpolarized ^{13}C MRA Using TrueFISP. *Magn Reson Med*. 2003; 50:256–262. DOI: 10.1002/mrm.10530 [PubMed: 12876701]
118. Yen Y-F, Le Roux P, Mayer D, King R, Spielman D, Tropp J, Butts Pauly K, Pfefferbaum A, Vasanawala S, Hurd R. T_2 relaxation times of ^{13}C metabolites in a rat hepatocellular carcinoma model measured *in vivo* using ^{13}C -MRS of hyperpolarized $[1-^{13}\text{C}]$ pyruvate. *NMR Biomed*. 2010; :n/a–n/a. DOI: 10.1002/nbm.1481
119. Reed GD, von Morze C, Bok R, Koelsch BL, Van Criekinge M, Smith KJ, Shang Hong, Larson PEZ, Kurhanewicz J, Vigneron DB. High Resolution ^{13}C MRI With Hyperpolarized Urea: In Vivo Mapping and ^{15}N Labeling Effects. *IEEE Trans Med Imaging*. 2014; 33:362–371. DOI: 10.1109/TMI.2013.2285120 [PubMed: 24235273]
120. Laustsen C, Stokholm Nørtinger T, Christoffer Hansen D, Qi H, Mose Nielsen P, Bonde Bertelsen L, Henrik Ardenkjaer-Larsen J, Stødkilde Jørgensen H. Hyperpolarized ^{13}C urea relaxation mechanism reveals renal changes in diabetic nephropathy: $[^{13}\text{C}]$ Urea Apparent Relaxation Contrast Reveals Diabetic Nephropathy. *Magn Reson Med*. 2016; 75:515–518. DOI: 10.1002/mrm.26036 [PubMed: 26584247]
121. Keshari KR, Wilson DM. Chemistry and biochemistry of ^{13}C hyperpolarized magnetic resonance using dynamic nuclear polarization. *Chem Soc Rev*. 2014; 43:1627. doi: 10.1039/c3cs60124b [PubMed: 24363044]
122. Golman K, in 't Zandt R, Thaning M. Real-time metabolic imaging. *Proc Natl Acad Sci*. 2006; 103:11270–11275. DOI: 10.1073/pnas.0601319103 [PubMed: 16837573]
123. Harris T, Eliyahu G, Frydman L, Degani H. Kinetics of hyperpolarized ^{13}C 1-pyruvate transport and metabolism in living human breast cancer cells. *Proc Natl Acad Sci*. 2009; 106:18131–18136. [PubMed: 19826085]
124. Shaghaghi H, Kadlec S, Deshpande C, Siddiqui S, Martinez D, Pourfathi M, Hamedani H, Ishii M, Profka H, Rizi R. Metabolic spectroscopy of inflammation in a bleomycin-induced lung injury model using hyperpolarized $1-^{13}\text{C}$ pyruvate. *NMR Biomed*. 2014; 27:939–947. DOI: 10.1002/nbm.3139 [PubMed: 24865640]
125. Park I, Larson PEZ, Tropp JL, Carvajal L, Reed G, Bok R, Robb F, Bringas J, Kells A, Pivrotto P, Bankiewicz K, Vigneron DB, Nelson SJ. Dynamic hyperpolarized carbon-13 MR metabolic imaging of nonhuman primate brain. *Magn Reson Med*. 2014; 71:19–25. DOI: 10.1002/mrm.25003 [PubMed: 24346964]
126. Spielman DM, Mayer D, Yen Y-F, Tropp J, Hurd RE, Pfefferbaum A. In vivo measurement of ethanol metabolism in the rat liver using magnetic resonance spectroscopy of hyperpolarized $[1-^{13}\text{C}]$ pyruvate. *Magn Reson Med*. 2009; 62:307–313. DOI: 10.1002/mrm.21998 [PubMed: 19526498]
127. Mulkern RV, Panych LP. Echo planar spectroscopic imaging. *Concepts Magn Reson*. 2001; 13:213–237. DOI: 10.1002/cmr.1011
128. Cunningham CH, Chen AP, Lustig M, Hargreaves BA, Lupo J, Xu D, Kurhanewicz J, Hurd RE, Pauly JM, Nelson SJ, Vigneron DB. Pulse sequence for dynamic volumetric imaging of hyperpolarized metabolic products. *J Magn Reson*. 2008; 193:139–146. DOI: 10.1016/j.jmr.2008.03.012 [PubMed: 18424203]
129. Cunningham CH, Chen AP, Albers MJ, Kurhanewicz J, Hurd RE, Yen Y-F, Pauly JM, Nelson SJ, Vigneron DB. Double spin-echo sequence for rapid spectroscopic imaging of hyperpolarized ^{13}C . *J Magn Reson*. 2007; 187:357–362. DOI: 10.1016/j.jmr.2007.05.014 [PubMed: 17562376]

130. Mayer D, Yen Y-F, Tropp J, Pfefferbaum A, Hurd RE, Spielman DM. Application of subsecond spiral chemical shift imaging to real-time multislice metabolic imaging of the rat in vivo after injection of hyperpolarized $^{13}\text{C}_1$ -pyruvate. *Magn Reson Med*. 2009; 62:557–564. DOI: 10.1002/mrm.22041 [PubMed: 19585607]
131. Mayer D, Yen Y-F, Levin YS, Tropp J, Pfefferbaum A, Hurd RE, Spielman DM. In vivo application of sub-second spiral chemical shift imaging (CSI) to hyperpolarized ^{13}C metabolic imaging: Comparison with phase-encoded CSI. *J Magn Reson*. 2010; 204:340–345. DOI: 10.1016/j.jmr.2010.03.005 [PubMed: 20346717]
132. Jiang W, Lustig M, Larson PEZ. Concentric rings K-space trajectory for hyperpolarized ^{13}C MR spectroscopic imaging. *Magn Reson Med*. 2014; :n/a–n/a. DOI: 10.1002/mrm.25577
133. Dixon WT. Simple proton spectroscopic imaging. *Radiology*. 1984; 153:189–194. [PubMed: 6089263]
134. Reeder SB, Wen Z, Yu H, Pineda AR, Gold GE, Markl M, Pelc NJ. Multicoil Dixon chemical species separation with an iterative least-squares estimation method. *Magn Reson Med*. 2004; 51:35–45. DOI: 10.1002/mrm.10675 [PubMed: 14705043]
135. Reeder SB, Brittain JH, Grist TM, Yen Y-F. Least-squares chemical shift separation for ^{13}C metabolic imaging. *J Magn Reson Imaging*. 2007; 26:1145–1152. DOI: 10.1002/jmri.21089 [PubMed: 17896366]
136. Wiens CN, Friesen-Waldner LJ, Wade TP, Sinclair KJ, McKenzie CA. Chemical shift encoded imaging of hyperpolarized ^{13}C pyruvate: Chemical Shift Encoded Imaging of ^{13}C Pyruvate. *Magn Reson Med*. 2015; 74:1682–1689. DOI: 10.1002/mrm.25532 [PubMed: 25427313]
137. Wiesinger F, Weidl E, Menzel MI, Janich MA, Khagai O, Glaser SJ, Haase A, Schwaiger M, Schulte RF. IDEAL spiral CSI for dynamic metabolic MR imaging of hyperpolarized $[1-^{13}\text{C}]$ pyruvate. *Magn Reson Med*. 2012; 68:8–16. DOI: 10.1002/mrm.23212 [PubMed: 22127962]
138. Sigfridsson A, Weiss K, Wissmann L, Busch J, Krajewski M, Batel M, Batsios G, Ernst M, Kozerke S. Hybrid multiband excitation multiecho acquisition for hyperpolarized ^{13}C spectroscopic imaging: Hybrid Multiband Excitation Multiecho Acquisition Metabolic Imaging. *Magn Reson Med*. 2014; :n/a–n/a. DOI: 10.1002/mrm.25294
139. Larson PEZ, Kerr AB, Chen AP, Lustig MS, Zierhut ML, Hu S, Cunningham CH, Pauly JM, Kurhanewicz J, Vigneron DB. Multiband excitation pulses for hyperpolarized ^{13}C dynamic chemical-shift imaging. *J Magn Reson*. 2008; 194:121–127. DOI: 10.1016/j.jmr.2008.06.010 [PubMed: 18619875]
140. Lau, AZ. Frequency-selective methods for hyperpolarized ^{13}C cardiac magnetic resonance imaging. University of Toronto; 2012. <https://tspace.library.utoronto.ca/handle/1807/34779> [accessed July 7, 2016]
141. Xing Y, Reed GD, Pauly JM, Kerr AB, Larson PEZ. Optimal variable flip angle schemes for dynamic acquisition of exchanging hyperpolarized substrates. *J Magn Reson*. 2013; 234:75–81. DOI: 10.1016/j.jmr.2013.06.003 [PubMed: 23845910]
142. Marco-Rius I, Cao P, von Morze C, Merritt M, Moreno KX, Chang G-Y, Ohliger MA, Pearce D, Kurhanewicz J, Larson PEZ, Vigneron DB. Multiband spectral-spatial RF excitation for hyperpolarized $[2-^{13}\text{C}]$ dihydroxyacetone ^{13}C -MR metabolism studies: Spectral-Spatial Excitation of HP ^{13}C -Dihydroxyacetone. *Magn Reson Med*. 2016; :n/a–n/a. DOI: 10.1002/mrm.26226
143. Gordon JW, Vigneron DB, Larson PEZ. Development of a symmetric echo planar imaging framework for clinical translation of rapid dynamic hyperpolarized ^{13}C imaging: Rapid Imaging of Hyperpolarized ^{13}C with Symmetric EPI. *Magn Reson Med*. 2016; :n/a–n/a. DOI: 10.1002/mrm.26123
144. Miller JJ, Lau AZ, Teh I, Schneider JE, Kinches P, Smart S, Ball V, Sibson NR, Tyler DJ. Robust and high resolution hyperpolarized metabolic imaging of the rat heart at 7 t with 3d spectral-spatial EPI: Hyperpolarized Cardiac Imaging with 3D Spectral-Spatial EPI. *Magn Reson Med*. 2016; 75:1515–1524. DOI: 10.1002/mrm.25730 [PubMed: 25991606]
145. Lau AZ, Miller JJ, Tyler DJ. Mapping of intracellular pH in the in vivo rodent heart using hyperpolarized $[1-^{13}\text{C}]$ pyruvate: Cardiac Intracellular pH Mapping. *Magn Reson Med*. 2016; doi: 10.1002/mrm.26260

146. Wang J, Wright AJ, Hu D, Hesketh R, Brindle KM. Single shot three-dimensional pulse sequence for hyperpolarized ^{13}C MRI: Fast 3D Hyperpolarized ^{13}C Imaging. *Magn Reson Med*. 2016; :n/a–n/a. DOI: 10.1002/mrm.26168
147. Chen AP, Leung K, Lam W, Hurd RE, Vigneron DB, Cunningham CH. Design of spectral-spatial outer volume suppression RF pulses for tissue specific metabolic characterization with hyperpolarized ^{13}C pyruvate. *J Magn Reson*. 2009; 200:344–348. DOI: 10.1016/j.jmr.2009.06.021 [PubMed: 19616981]
148. von Morze C, Reed G, Shin P, Larson PEZ, Hu S, Bok R, Vigneron DB. Multi-band frequency encoding method for metabolic imaging with hyperpolarized $[1-^{13}\text{C}]$ pyruvate. *J Magn Reson*. 2011; 211:109–113. DOI: 10.1016/j.jmr.2011.04.007 [PubMed: 21596601]
149. Ghosh RK, Kadlecik SJ, Pourfathi M, Rizi RR. Efficient production of hyperpolarized bicarbonate by chemical reaction on a DNP precursor to measure pH. *Magn Reson Med*. 2014; :n/a–n/a. DOI: 10.1002/mrm.25530
150. Ramirez MS, Lee J, Walker CM, Sandulache VC, Hennel F, Lai SY, Bankson JA. Radial spectroscopic MRI of hyperpolarized $[1-^{13}\text{C}]$ pyruvate at 7 tesla: Radial MRI of Hyperpolarized Pyruvate at 7T. *Magn Reson Med*. 2014; 72:986–995. DOI: 10.1002/mrm.25004 [PubMed: 24186845]
151. Mayer D, Levin YS, Hurd RE, Glover GH, Spielman DM. Fast metabolic imaging of systems with sparse spectra: Application for hyperpolarized ^{13}C imaging. *Magn Reson Med*. 2006; 56:932–937. DOI: 10.1002/mrm.21025 [PubMed: 16941617]
152. Yang S, Lee J, Joe E, Lee H, Choi Y-S, Park JM, Spielman D, Song H-T, Kim D-H. Metabolite-selective hyperpolarized ^{13}C imaging using extended chemical shift displacement at 9.4T. *Magn Reson Imaging*. 2016; 34:535–540. DOI: 10.1016/j.mri.2015.12.023 [PubMed: 26707851]
153. Golman K, Olsson LE, Axelsson O, Månsson S, Karlsson M, Petersson JS. Molecular imaging using hyperpolarized ^{13}C . *Br J Radiol*. 2003; 76:S118–S127. DOI: 10.1259/bjr/26631666 [PubMed: 15572334]
154. von Morze C, Bok RA, Reed GD, Ardenkjaer-Larsen JH, Kurhanewicz J, Vigneron DB. Simultaneous multiagent hyperpolarized ^{13}C perfusion imaging: Multiagent HP ^{13}C Perfusion MRI. *Magn Reson Med*. 2014; 72:1599–1609. DOI: 10.1002/mrm.25071 [PubMed: 24382698]
155. von Morze C, Sukumar S, Reed GD, Larson PEZ, Bok RA, Kurhanewicz J, Vigneron DB. Frequency-specific SSFP for hyperpolarized ^{13}C metabolic imaging at 14.1 T. *Magn Reson Imaging*. 2013; 31:163–170. DOI: 10.1016/j.mri.2012.06.037 [PubMed: 22898680]
156. Arunachalam A, Whitt D, Fish K, Giaquinto R, Piel J, Watkins R, Hancu I. Accelerated spectroscopic imaging of hyperpolarized C-13 pyruvate using SENSE parallel imaging. *NMR Biomed*. 2009; 22:867–873. DOI: 10.1002/nbm.1401 [PubMed: 19489035]
157. Shin PJ, Larson PEZ, Uecker M, Reed GD, Kerr AB, Tropp J, Ohliger MA, Nelson SJ, Pauly JM, Lustig M, Vigneron DB. Chemical shift separation with controlled aliasing for hyperpolarized ^{13}C metabolic imaging: Accelerated Metabolite Imaging in Hyperpolarized ^{13}C MR. *Magn Reson Med*. 2015; 74:978–989. DOI: 10.1002/mrm.25473 [PubMed: 25298086]
158. Cao P, Shin PJ, Park I, Najac C, Marco-Rius I, Vigneron DB, Nelson SJ, Ronen SM, Larson PEZ. Accelerated high-bandwidth MR spectroscopic imaging using compressed sensing: Random Walk MRSI. *Magn Reson Med*. 2016; doi: 10.1002/mrm.26272
159. Geraghty BJ, Lau JYC, Chen AP, Cunningham CH. Accelerated 3D echo-planar imaging with compressed sensing for time-resolved hyperpolarized ^{13}C studies: Compressed Sensing ^{13}C 3D EPI. *Magn Reson Med*. 2016; :n/a–n/a. DOI: 10.1002/mrm.26125
160. Hu S, Lustig M, Balakrishnan A, Larson PEZ, Bok R, Kurhanewicz J, Nelson SJ, Goga A, Pauly JM, Vigneron DB. 3D compressed sensing for highly accelerated hyperpolarized ^{13}C MRSI with in vivo applications to transgenic mouse models of cancer. *Magn Reson Med*. 2010; 63:312–321. DOI: 10.1002/mrm.22233 [PubMed: 20017160]
161. Schroeder MA, Cochlin LE, Heather LC, Clarke K, Radda GK, Tyler DJ. In vivo assessment of pyruvate dehydrogenase flux in the heart using hyperpolarized carbon-13 magnetic resonance. *Proc Natl Acad Sci*. 2008; 105:12051–12056. DOI: 10.1073/pnas.0805953105 [PubMed: 18689683]

162. Seth P, Grant A, Tang J, Vinogradov E, Wang X, Lenkinski R, Sukhatme VP. On-target Inhibition of Tumor Fermentative Glycolysis as Visualized by Hyperpolarized Pyruvate. *Neoplasia*. 2011; 13:60–71. DOI: 10.1593/neo.101020 [PubMed: 21245941]
163. Hill DK, Orton MR, Mariotti E, Boulton JKR, Panek R, Jafar M, Parkes HG, Jamin Y, Miniotti MF, Al-Saffar NMS, Belouche-Babari M, Robinson SP, Leach MO, Chung Y-L, Eyrkyn TR. Model Free Approach to Kinetic Analysis of Real-Time Hyperpolarized ¹³C Magnetic Resonance Spectroscopy Data. *PLOS ONE*. 2013; 8:e71996.doi: 10.1371/journal.pone.0071996 [PubMed: 24023724]
164. Shaghghi H, Kadlecsek S, Siddiqui S, Pourfathi M, Hamedani H, Clapp J, Profka H, Rizi R. Ascorbic acid prolongs the viability and stability of isolated perfused lungs: A mechanistic study using ³¹P and hyperpolarized ¹³C nuclear magnetic resonance. *Free Radic Biol Med*. 2015; 89:62–71. DOI: 10.1016/j.freeradbiomed.2015.06.042 [PubMed: 26165188]
165. Zierhut ML, Yen Y-F, Chen AP, Bok R, Albers MJ, Zhang V, Tropp J, Park I, Vigneron DB, Kurhanewicz J, Hurd RE, Nelson SJ. Kinetic modeling of hyperpolarized ¹³C1-pyruvate metabolism in normal rats and TRAMP mice. *J Magn Reson*. 2010; 202:85–92. DOI: 10.1016/j.jmr.2009.10.003 [PubMed: 19884027]
166. Li LZ, Kadlecsek S, Xu HN, Daye D, Pullinger B, Profka H, Chodosh L, Rizi R. Ratiometric analysis in hyperpolarized NMR (I): test of the two-site exchange model and the quantification of reaction rate constants: RATIOMETRIC DATA ANALYSIS FOR HYPERPOLARIZED NMR. *NMR Biomed*. 2013; 26:1308–1320. DOI: 10.1002/nbm.2953 [PubMed: 23625472]
167. Kadlecsek S, Shaghghi H, Siddiqui S, Profka H, Pourfathi M, Rizi R. The effect of exogenous substrate concentrations on true and apparent metabolism of hyperpolarized pyruvate in the isolated perfused lung. *NMR Biomed*. 2014; 27:1557–1570. DOI: 10.1002/nbm.3219 [PubMed: 25330438]
168. Yoshihara HAI, Bastiaansen JAM, Berthonneche C, Comment A, Schwitter J. An Intact Small Animal Model of Myocardial Ischemia-Reperfusion: Characterization of Metabolic Changes by Hyperpolarized ¹³C MR Spectroscopy. *Am J Physiol - Heart Circ Physiol*. 2015; ajpheart.00376.2015. doi: 10.1152/ajpheart.00376.2015
169. Zewe V, Fromm HJ. Kinetic studies of rabbit muscle lactate dehydrogenase. *J Biol Chem*. 1962; 237:1668–1675. [PubMed: 14010189]
170. Borgmann U, Laidler KJ, Moon TW. Kinetics and thermodynamics of lactate dehydrogenases from beef heart, beef muscle, and flounder muscle. *Can J Biochem*. 1975; 53:1196–1206. [PubMed: 1192261]
171. Golman K, Zandt Ri, Lerche M, Pehrson R, Ardenkjaer-Larsen JH. Metabolic Imaging by Hyperpolarized ¹³C Magnetic Resonance Imaging for In vivo Tumor Diagnosis. *Cancer Res*. 2006; 66:10855–10860. DOI: 10.1158/0008-5472.CAN-06-2564 [PubMed: 17108122]
172. Chen AP, Albers MJ, Cunningham CH, Kohler SJ, Yen Y-F, Hurd RE, Tropp J, Bok R, Pauly JM, Nelson SJ, Kurhanewicz J, Vigneron DB. Hyperpolarized C-13 spectroscopic imaging of the TRAMP mouse at 3T—Initial experience. *Magn Reson Med*. 2007; 58:1099–1106. DOI: 10.1002/mrm.21256 [PubMed: 17969006]
173. Albers MJ, Bok R, Chen AP, Cunningham CH, Zierhut ML, Zhang VY, Kohler SJ, Tropp J, Hurd RE, Yen Y-F, Nelson SJ, Vigneron DB, Kurhanewicz J. Hyperpolarized ¹³C Lactate, Pyruvate, and Alanine: Noninvasive Biomarkers for Prostate Cancer Detection and Grading. *Cancer Res*. 2008; 68:8607–8615. DOI: 10.1158/0008-5472.CAN-08-0749 [PubMed: 18922937]
174. Pullinger B, Profka H, Ardenkjaer-Larsen JH, Kuzma NN, Kadlecsek S, Rizi RR. Metabolism of hyperpolarized [1-¹³C]pyruvate in the isolated perfused rat lung - an ischemia study: MEASUREMENTS OF LUNG METABOLISM USING HYPERPOLARIZED 1-¹³C PYRUVATE. *NMR Biomed*. 2012; 25:1113–1118. DOI: 10.1002/nbm.2777 [PubMed: 22311307]
175. Hu S, Chen AP, Zierhut ML, Bok R, Yen Y-F, Schroeder MA, Hurd RE, Nelson SJ, Kurhanewicz J, Vigneron DB. In Vivo Carbon-13 Dynamic MRS and MRSI of Normal and Fasted Rat Liver with Hyperpolarized ¹³C-Pyruvate. *Mol Imaging Biol*. 2009; 11:399–407. DOI: 10.1007/s11307-009-0218-z [PubMed: 19424761]
176. Maren TH. Carbonic anhydrase: chemistry, physiology, and inhibition. *Physiol Rev*. 1967; 47:595–781. [PubMed: 4964060]

177. Merritt ME, Harrison C, Storey C, Jeffrey FM, Sherry AD, Malloy CR. Hyperpolarized ^{13}C allows a direct measure of flux through a single enzyme-catalyzed step by NMR. *Proc Natl Acad Sci*. 2007; 104:19773–19777. DOI: 10.1073/pnas.0706235104 [PubMed: 18056642]
178. Merritt ME, Harrison C, Sherry AD, Malloy CR, Burgess SC. Flux through hepatic pyruvate carboxylase and phosphoenolpyruvate carboxykinase detected by hyperpolarized ^{13}C magnetic resonance. *Proc Natl Acad Sci*. 2011; 108:19084–19089. DOI: 10.1073/pnas.1111247108 [PubMed: 22065779]
179. Moreno KX, Sabelhaus SM, Merritt ME, Sherry AD, Malloy CR. Competition of pyruvate with physiological substrates for oxidation by the heart: implications for studies with hyperpolarized $[1-^{13}\text{C}]$ pyruvate. *Am J Physiol - Heart Circ Physiol*. 2010; 298:H1556–H1564. DOI: 10.1152/ajpheart.00656.2009 [PubMed: 20207817]
180. Marja ska M, Iltis I, Shestov AA, Deelchand DK, Nelson C, Urbil K, Henry P-G. In vivo ^{13}C spectroscopy in the rat brain using hyperpolarized $[1-^{13}\text{C}]$ pyruvate and $[2-^{13}\text{C}]$ pyruvate. *J Magn Reson*. 2010; 206:210–218. DOI: 10.1016/j.jmr.2010.07.006 [PubMed: 20685141]
181. Schroeder MA, Atherton HJ, Ball DR, Cole MA, Heather LC, Griffin JL, Clarke K, Radda GK, Tyler DJ. Real-time assessment of Krebs cycle metabolism using hyperpolarized ^{13}C magnetic resonance spectroscopy. *FASEB J*. 2009; 23:2529–2538. DOI: 10.1096/fj.09-129171 [PubMed: 19329759]
182. Hu S, Yoshihara HAI, Bok R, Zhou J, Zhu M, Kurhanewicz J, Vigneron DB. Use of hyperpolarized $[1-^{13}\text{C}]$ pyruvate and $[2-^{13}\text{C}]$ pyruvate to probe the effects of the anticancer agent dichloroacetate on mitochondrial metabolism in vivo in the normal rat. *Magn Reson Imaging*. 2012; 30:1367–1372. DOI: 10.1016/j.mri.2012.05.012 [PubMed: 22819176]
183. Schroeder MA, Atherton HJ, Dodd MS, Lee P, Cochlin LE, Radda GK, Clarke K, Tyler DJ. The Cycling of Acetyl-Coenzyme A Through Acetylcarnitine Buffers Cardiac Substrate Supply A Hyperpolarized ^{13}C Magnetic Resonance Study. *Circ Cardiovasc Imaging*. 2012; 5:201–209. DOI: 10.1161/CIRCIMAGING.111.969451 [PubMed: 22238215]
184. Bastiaansen JAM, Cheng T, Mishkovsky M, Duarte JMN, Comment A, Gruetter R. In vivo enzymatic activity of acetylCoA synthetase in skeletal muscle revealed by ^{13}C turnover from hyperpolarized $[1-^{13}\text{C}]$ acetate to $[1-^{13}\text{C}]$ acetylcarnitine. *Biochim Biophys Acta BBA - Gen Subj*. 2013; 1830:4171–4178. DOI: 10.1016/j.bbagen.2013.03.023
185. Flori A, Liserani M, Frijia F, Giovannetti G, Lionetti V, Casieri V, Positano V, Aquaro GD, Recchia FA, Santarelli MF, Landini L, Ardenkjaer-Larsen JH, Menichetti L. Real-time cardiac metabolism assessed with hyperpolarized $[1-^{13}\text{C}]$ acetate in a large-animal model: $[^{13}\text{C}]$ ACETATE FOR REAL-TIME CARDIAC METABOLISM. *Contrast Media Mol Imaging*. 2015; 10:194–202. DOI: 10.1002/cmmi.1618 [PubMed: 25201079]
186. Hu S, Zhu M, Yoshihara HAI, Wilson DM, Keshari KR, Shin P, Reed G, von Morze C, Bok R, Larson PEZ, Kurhanewicz J, Vigneron DB. In vivo measurement of normal rat intracellular pyruvate and lactate levels after injection of hyperpolarized $[1-^{13}\text{C}]$ alanine. *Magn Reson Imaging*. 2011; 29:1035–1040. DOI: 10.1016/j.mri.2011.07.001 [PubMed: 21855243]
187. Felig P. The glucose-alanine cycle. *Metabolism*. 1973; 22:179–207. DOI: 10.1016/0026-0495(73)90269-2 [PubMed: 4567003]
188. Hata R, Nonaka H, Takakusagi Y, Ichikawa K, Sando S. Design of a Hyperpolarized Molecular Probe for Detection of Aminopeptidase N Activity. *Angew Chem*. 2016; 128:1797–1800. DOI: 10.1002/ange.201509457
189. Keshari KR, Sai V, Wang ZJ, VanBrocklin HF, Kurhanewicz J, Wilson DM. Hyperpolarized $[1-^{13}\text{C}]$ Dehydroascorbate MR Spectroscopy in a Murine Model of Prostate Cancer: Comparison with ^{18}F -FDG PET. *J Nucl Med*. 2013; jnumed.112.115402. doi: 10.2967/jnumed.112.115402
190. Willis RJ, Kratzing CC. Transport of ascorbic acid in perfused rat lung. *Pflüg Arch*. 1975; 356:93–98.
191. Du J, Cullen JJ, Buettner GR. Ascorbic acid: Chemistry, biology and the treatment of cancer. *Biochim Biophys Acta BBA - Rev Cancer*. 2012; 1826:443–457. DOI: 10.1016/j.bbcan.2012.06.003
192. Bohndiek SE, Kettunen MI, Hu D, Kennedy BWC, Boren J, Gallagher FA, Brindle KM. Hyperpolarized $[1-^{13}\text{C}]$ -Ascorbic and Dehydroascorbic Acid: Vitamin C as a Probe for Imaging

Redox Status in Vivo. *J Am Chem Soc.* 2011; 133:11795–11801. DOI: 10.1021/ja2045925 [PubMed: 21692446]

193. Keshari KR, Kurhanewicz J, Bok R, Larson PEZ, Vigneron DB, Wilson DM. Hyperpolarized ¹³C dehydroascorbate as an endogenous redox sensor for in vivo metabolic imaging. *Proc Natl Acad Sci.* 2011; 108:18606–18611. DOI: 10.1073/pnas.1106920108 [PubMed: 22042839]
194. Fraser ME, James MN, Bridger WA, Wolodko WT. A detailed structural description of *Escherichia coli* succinyl-CoA synthetase. *J Mol Biol.* 1999; 285:1633–1653. [PubMed: 9917402]
195. Oyedotun KS, Lemire BD. The Quaternary Structure of the *Saccharomyces cerevisiae* Succinate Dehydrogenase: HOMOLOGUE MODELING, COFACTOR DOCKING, AND MOLECULAR DYNAMICS SIMULATION STUDIES. *J Biol Chem.* 2004; 279:9424–9431. DOI: 10.1074/jbc.M311876200 [PubMed: 14672929]
196. Ishii N, Fujii M, Hartman PS, Tsuda M, Yasuda K, Senoo-Matsuda N, Yanase S, Ayusawa D, Suzuki K. A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and ageing in nematodes. *Nature.* 1998; 394:694–697. [PubMed: 9716135]
197. Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, Pan Y, Simon MC, Thompson CB, Gottlieb E. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF- α prolyl hydroxylase. *Cancer Cell.* 2005; 7:77–85. DOI: 10.1016/j.ccr.2004.11.022 [PubMed: 15652751]
198. Hövener J-B, Chekmenev EY, Harris KC, Perman WH, Tran TT, Ross BD, Bhattacharya P. Quality assurance of PASADENA hyperpolarization for ¹³C biomolecules. *Magn Reson Mater Phys Biol Med.* 2008; 22:123–134. DOI: 10.1007/s10334-008-0154-y
199. Billingsley KL, Josan S, Park JM, Tee SS, Spielman-Sun E, Hurd R, Mayer D, Spielman D. Hyperpolarized [1,4-¹³C]-diethylsuccinate: a potential DNP substrate for *in vivo* metabolic imaging: HYPERPOLARIZED DIETHYLSUCCINATE: A DNP SUBSTRATE FOR IN VIVO IMAGING. *NMR Biomed.* 2014; 27:356–362. DOI: 10.1002/nbm.3071 [PubMed: 24421249]
200. Gallagher FA, Kettunen MI, Day SE, Hu D-E, Ardenkjær-Larsen JH, in 't Zandt R, Jensen PR, Karlsson M, Golman K, Lerche MH, Brindle KM. Magnetic resonance imaging of pH in vivo using hyperpolarized ¹³C-labelled bicarbonate. *Nature.* 2008; 453:940–943. DOI: 10.1038/nature07017 [PubMed: 18509335]
201. Kottmann RM, Kulkarni AA, Smolnycki KA, Lyda E, Dahanayake T, Salibi R, Honnons S, Jones C, Isern NG, Hu JZ, Nathan SD, Grant G, Phipps RP, Sime PJ. Lactic Acid Is Elevated in Idiopathic Pulmonary Fibrosis and Induces Myofibroblast Differentiation via pH-Dependent Activation of Transforming Growth Factor- β . *Am J Respir Crit Care Med.* 2012; 186:740–751. DOI: 10.1164/rccm.201201-0084OC [PubMed: 22923663]
202. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. *Cell.* 2011; 144:646–674. DOI: 10.1016/j.cell.2011.02.013 [PubMed: 21376230]
203. Harris T, Degani H, Frydman L. Hyperpolarized ¹³C NMR studies of glucose metabolism in living breast cancer cell cultures: HYPERPOLARIZED ¹³C GLUCOSE NMR OF LIVING BREAST CANCER CELL METABOLISM. *NMR Biomed.* 2013; 26:1831–1843. DOI: 10.1002/nbm.3024 [PubMed: 24115045]
204. Timm KN, Hartl J, Keller MA, Hu D-E, Kettunen MI, Rodrigues TB, Ralser M, Brindle KM. Hyperpolarized [U-2H, U-¹³C]Glucose reports on glycolytic and pentose phosphate pathway activity in EL4 tumors and glycolytic activity in yeast cells. *Magn Reson Med.* 2014; :n/a–n/a. DOI: 10.1002/mrm.25561
205. Kurhanewicz J, Vigneron DB, Brindle K, Chekmenev EY, Comment A, Cunningham CH, DeBerardinis RJ, Green GG, Leach MO, Rajan SS, Rizi RR, Ross BD, Warren WS, Malloy CR. Analysis of Cancer Metabolism by Imaging Hyperpolarized Nuclei: Prospects for Translation to Clinical Research. *Neoplasia.* 2011; 13:81–97. DOI: 10.1593/neo.101102 [PubMed: 21403835]
206. Dodd MS, Ball V, Bray R, Ashrafian H, Watkins H, Clarke K, Tyler DJ. In vivo mouse cardiac hyperpolarized magnetic resonance spectroscopy. *J Cardiovasc Magn Reson.* 2013; 15:19. [PubMed: 23414451]
207. Ball DR, Rowlands B, Dodd MS, Le Page L, Ball V, Carr CA, Clarke K, Tyler DJ. Hyperpolarized butyrate: A metabolic probe of short chain fatty acid metabolism in the heart. *Magn Reson Med.* 2014; 71:1663–1669. DOI: 10.1002/mrm.24849 [PubMed: 23798473]

208. Mayer D, Yen Y-F, Josan S, Park JM, Pfefferbaum A, Hurd RE, Spielman DM. Application of hyperpolarized [1-¹³C]lactate for the in vivo investigation of cardiac metabolism: INVESTIGATION OF CARDIAC METABOLISM WITH HYPERPOLARIZED [1-¹³C]LACTATE. *NMR Biomed.* 2012; 25:1119–1124. DOI: 10.1002/nbm.2778 [PubMed: 22278751]
209. Atherton HJ, Dodd MS, Heather LC, Schroeder MA, Griffin JL, Radda GK, Clarke K, Tyler DJ. Role of Pyruvate Dehydrogenase Inhibition in the Development of Hypertrophy in the Hyperthyroid Rat Heart A Combined Magnetic Resonance Imaging and Hyperpolarized Magnetic Resonance Spectroscopy Study. *Circulation.* 2011; 123:2552–2561. DOI: 10.1161/CIRCULATIONAHA.110.011387 [PubMed: 21606392]
210. Bastiaansen JAM, Cheng T, Lei H, Gruetter R, Comment A. Direct noninvasive estimation of myocardial tricarboxylic acid cycle flux in vivo using hyperpolarized ¹³C magnetic resonance. *J Mol Cell Cardiol.* 2015; 87:129–137. DOI: 10.1016/j.yjmcc.2015.08.012 [PubMed: 26297113]
211. Koellisch U, Gringeri CV, Rancan G, Farell EV, Menzel MI, Haase A, Schwaiger M, Schulte RF. Metabolic imaging of hyperpolarized [1-¹³C]acetate and [1-¹³C]acetylcarnitine - investigation of the influence of dobutamine induced stress: Metabolic Imaging of Acetate and Acetylcarnitine. *Magn Reson Med.* 2015; 74:1011–1018. DOI: 10.1002/mrm.25485 [PubMed: 25298189]
212. Merritt ME, Harrison C, Storey C, Sherry AD, Malloy CR. Inhibition of carbohydrate oxidation during the first minute of reperfusion after brief ischemia: NMR detection of hyperpolarized ¹³CO₂ and H ¹³CO₃-. *Magn Reson Med.* 2008; 60:1029–1036. DOI: 10.1002/mrm.21760 [PubMed: 18956454]
213. Golman K, Petersson JS, Magnusson P, Johansson E, Åkeson P, Chai C-M, Hansson G, Månsson S. Cardiac metabolism measured noninvasively by hyperpolarized ¹³C MRI. *Magn Reson Med.* 2008; 59:1005–1013. DOI: 10.1002/mrm.21460 [PubMed: 18429038]
214. Schroeder MA, Swietach P, Atherton HJ, Gallagher FA, Lee P, Radda GK, Clarke K, Tyler DJ. Measuring intracellular pH in the heart using hyperpolarized carbon dioxide and bicarbonate: a ¹³C and ³¹P magnetic resonance spectroscopy study. *Cardiovasc Res.* 2010; 86:82–91. DOI: 10.1093/cvr/cvp396 [PubMed: 20008827]
215. Kerbey AL, Randle PJ, Cooper RH, Whitehouse S, Pask HT, Denton RM. Regulation of pyruvate dehydrogenase in rat heart. Mechanism of regulation of proportions of dephosphorylated and phosphorylated enzyme by oxidation of fatty acids and ketone bodies and of effects of diabetes: role of coenzyme A, acetyl-coenzyme A and reduced and oxidized nicotinamide-adenine dinucleotide. *Biochem J.* 1976; 154:327–348. DOI: 10.1042/bj1540327 [PubMed: 180974]
216. Rui, L. Energy Metabolism in the Liver. In: Terjung, R., editor. *Compr Physiol.* John Wiley & Sons, Inc; Hoboken, NJ, USA: 2014. p. 177-197. <http://doi.wiley.com/10.1002/cphy.c130024> [accessed June 10, 2016]
217. Reichard GA, Moury NF, Hochella NJ, Patterson AL, Weinhouse S. Quantitative Estimation of the Cori Cycle in the Human. *J Biol Chem.* 1963; 238:495–501.
218. Lee P, Leong W, Tan T, Lim M, Han W, Radda GK. In Vivo hyperpolarized carbon-13 magnetic resonance spectroscopy reveals increased pyruvate carboxylase flux in an insulin-resistant mouse model. *Hepatology.* 2013; 57:515–524. DOI: 10.1002/hep.26028 [PubMed: 22911492]
219. Jensen PR, Peitersen T, Karlsson M, in 't Zandt R, Gisselsson A, Hansson G, Meier S, Lerche MH. Tissue-specific Short Chain Fatty Acid Metabolism and Slow Metabolic Recovery after Ischemia from Hyperpolarized NMR in Vivo. *J Biol Chem.* 2009; 284:36077–36082. DOI: 10.1074/jbc.M109.066407 [PubMed: 19861411]
220. CLIP. A new prognostic system for hepatocellular carcinoma: A retrospective study of 435 patients. *Hepatology.* 1998; 28:751–755. DOI: 10.1002/hep.510280322 [PubMed: 9731568]
221. Gallagher FA, Kettunen MI, Day SE, Lerche M, Brindle KM. ¹³C MR spectroscopy measurements of glutaminase activity in human hepatocellular carcinoma cells using hyperpolarized ¹³C-labeled glutamine. *Magn Reson Med.* 2008; 60:253–257. DOI: 10.1002/mrm.21650 [PubMed: 18666104]
222. Darpolor MM, Yen Y-F, Chua M-S, Xing L, Clarke-Katzenberg RH, Shi W, Mayer D, Josan S, Hurd RE, Pfefferbaum A, Senadheera L, So S, Hofmann LV, Glazer GM, Spielman DM. In vivo MRSI of hyperpolarized [1-¹³C]pyruvate metabolism in rat hepatocellular carcinoma. *NMR Biomed.* 2011; 24:506–513. DOI: 10.1002/nbm.1616 [PubMed: 21674652]

223. Darpolor MM. Human Hepatocellular Carcinoma Metabolism: Imaging by Hyperpolarized ^{13}C Magnetic Resonance Spectroscopy. *J Liver Dis Transplant*. 2012; 01doi: 10.4172/2325-9612.1000101
224. Bard-Chapeau EA, Nguyen A-T, Rust AG, Sayadi A, Lee P, Chua BQ, New L-S, de Jong J, Ward JM, Chin CKY, Chew V, Toh HC, Abastado J-P, Benoukraf T, Soong R, Bard FA, Dupuy AJ, Johnson RL, Radda GK, Chan ECY, Wessels LFA, Adams DJ, Jenkins NA, Copeland NG. Transposon mutagenesis identifies genes driving hepatocellular carcinoma in a chronic hepatitis B mouse model. *Nat Genet*. 2013; 46:24–32. DOI: 10.1038/ng.2847 [PubMed: 24316982]
225. Düwel S, Durst M, Gringeri CV, Kosanke Y, Gross C, Janich MA, Haase A, Glaser SJ, Schwaiger M, Schulte RF, Braren R, Menzel MI. Multiparametric human hepatocellular carcinoma characterization and therapy response evaluation by hyperpolarized ^{13}C MRSI: HCC Therapy Response Evaluation By Hyperpolarized ^{13}C MRSI. *NMR Biomed*. 2016; doi: 10.1002/nbm.3561
226. Kantarci K, Knopman DS, Dickson DW, Parisi JE, Whitwell JL, Weigand SD, Josephs KA, Boeve BF, Petersen RC, Jack CR. Alzheimer Disease: Postmortem Neuropathologic Correlates of Antemortem ^1H MR Spectroscopy Metabolite Measurements I. *Radiology*. 2008; 248:210–220. DOI: 10.1148/radiol.2481071590 [PubMed: 18566174]
227. Pan JW, Williamson A, Cavus I, Hetherington HP, Zaveri H, Petroff OAC, Spencer DD. Neurometabolism in human epilepsy. *Epilepsia*. 2008; 49:31–41. DOI: 10.1111/j.1528-1167.2008.01508.x [PubMed: 18304254]
228. Schellinger PD, Jansen O, Fiebach JB, Heiland S, Steiner T, Schwab S, Pohlers O, Ryssel H, Sartor K, Hacke W. Monitoring Intravenous Recombinant Tissue Plasminogen Activator Thrombolysis for Acute Ischemic Stroke With Diffusion and Perfusion MRI. *Stroke*. 2000; 31:1318–1328. DOI: 10.1161/01.STR.31.6.1318 [PubMed: 10835451]
229. Provenzale JM, Mukundan S, Barboriak DP. Diffusion-weighted and Perfusion MR Imaging for Brain Tumor Characterization and Assessment of Treatment Response. *Radiology*. 2006; 239:632–649. DOI: 10.1148/radiol.2393042031 [PubMed: 16714455]
230. Weber MA, Zoubaa S, Schlieter M, Jüttler E, Huttner HB, Geletneky K, Ittrich C, Lichy MP, Kroll A, Debus J, et al. Diagnostic performance of spectroscopic and perfusion MRI for distinction of brain tumors. *Neurology*. 2006; 66:1899–1906. [PubMed: 16801657]
231. Murphy-Boesch J, Stoyanova R, Srinivasan R, Willard T, Vigneron D, Nelson S, Taylor JS, Brown TR. Proton-decoupled ^3P chemical shift imaging of the human brain in normal volunteers. *NMR Biomed*. 1993; 6:173–180. DOI: 10.1002/nbm.1940060302 [PubMed: 8394101]
232. Henry P-G, Adriany G, Deelchand D, Gruetter R, Marjanska M, Öz G, Seaquist ER, Shestov A, Urbil K. In vivo ^{13}C NMR spectroscopy and metabolic modeling in the brain: a practical perspective. *Magn Reson Imaging*. 2006; 24:527–539. DOI: 10.1016/j.mri.2006.01.003 [PubMed: 16677959]
233. Nelson SJ, Graves E, Pirzkall A, Li X, Antiniw Chan A, Vigneron DB, McKnight TR. In vivo molecular imaging for planning radiation therapy of gliomas: An application of ^1H MRSI. *J Magn Reson Imaging*. 2002; 16:464–476. DOI: 10.1002/jmri.10183 [PubMed: 12353260]
234. Warburg O. On the Origin of Cancer Cells. *Science*. 1956; 123:309–314. [PubMed: 13298683]
235. He Q, Bhujwala ZM, Glickson JD. Proton detection of choline and lactate in EMT6 tumors by spin-echo-enhanced selective multiple-quantum-coherence transfer. *J Magn Reson B*. 1996; 112:18–25. [PubMed: 8661302]
236. Star-Lack J, Spielman D, Adalsteinsson E, Kurhanewicz J, Terris DJ, Vigneron DB. In Vivo Lactate Editing with Simultaneous Detection of Choline, Creatine, NAA, and Lipid Singlets at 1.5 T Using PRESS Excitation with Applications to the Study of Brain and Head and Neck Tumors. *J Magn Reson*. 1998; 133:243–254. DOI: 10.1006/jmre.1998.1458 [PubMed: 9716465]
237. Saraswathy S, Crawford FW, Lamborn KR, Pirzkall A, Chang S, Cha S, Nelson SJ. Evaluation of MR markers that predict survival in patients with newly diagnosed GBM prior to adjuvant therapy. *J Neurooncol*. 2008; 91:69–81. DOI: 10.1007/s11060-008-9685-3 [PubMed: 18810326]
238. Park I, Larson PEZ, Zierhut ML, Hu S, Bok R, Ozawa T, Kurhanewicz J, Vigneron DB, VandenBerg SR, James CD, Nelson SJ. Hyperpolarized ^{13}C magnetic resonance metabolic

- imaging: application to brain tumors. *Neuro-Oncol.* 2010; 12:133–144. DOI: 10.1093/neuonc/nop043 [PubMed: 20150380]
239. Kugel H, Heindel W, Ernestus RI, Bunke J, du Mesnil R, Friedmann G. Human brain tumors: spectral patterns detected with localized H-1 MR spectroscopy. *Radiology.* 1992; 183:701–709. DOI: 10.1148/radiology.183.3.1584924 [PubMed: 1584924]
 240. Davson, H. An introduction to the blood-brain barrier. CRC Press LLC; 1993.
 241. Ross B, Lin A, Harris K, Bhattacharya P, Schweinsburg B. Clinical experience with ¹³C MRS in vivo. *NMR Biomed.* 2003; 16:358–369. DOI: 10.1002/nbm.852 [PubMed: 14679500]
 242. Heron DE, Andrade RS, Beriwal S, Smith RP. PET-CT in Radiation Oncology: The Impact on Diagnosis, Treatment Planning, and Assessment of Treatment Response. *Am J Clin Oncol.* 2008; 31:352–362. DOI: 10.1097/COC.0b013e318162f150 [PubMed: 18845994]
 243. Facey K, Bradbury I, Laking G, Payne E. Overview of the clinical effectiveness of positron emission tomography imaging in selected cancers. *Health Technol Assess-Southampton.* 2007; 11 [accessed July 11, 2016] https://www.researchgate.net/profile/Karen_Facey/publication/5846787_Overview_of_the_clinical_effectiveness_of_positron_emission_tomography_imaging_in_selected_cancers/links/0912f513f9fba0c825000000.pdf.
 244. Kato T, Shinoda J, Nakayama N, Miwa K, Okumura A, Yano H, Yoshimura S, Maruyama T, Muragaki Y, Iwama T. Metabolic Assessment of Gliomas Using ¹¹C-Methionine, [¹⁸F] Fluorodeoxyglucose, and ¹¹C-Choline Positron-Emission Tomography. *Am J Neuroradiol.* 2008; 29:1176–1182. DOI: 10.3174/ajnr.A1008 [PubMed: 18388218]
 245. Pauleit D, Stoffels G, Bachofner A, Floeth FW, Sabel M, Herzog H, Tellmann L, Jansen P, Reifenberger G, Hamacher K, Coenen HH, Langen K-J. Comparison of ¹⁸F-FET and ¹⁸F-FDG PET in brain tumors. *Nucl Med Biol.* 2009; 36:779–787. DOI: 10.1016/j.nucmedbio.2009.05.005 [PubMed: 19720290]
 246. Park I, Bok R, Ozawa T, Phillips JJ, James CD, Vigneron DB, Ronen SM, Nelson SJ. Detection of early response to temozolomide treatment in brain tumors using hyperpolarized ¹³C MR metabolic imaging. *J Magn Reson Imaging.* 2011; 33:1284–1290. DOI: 10.1002/jmri.22563 [PubMed: 21590996]
 247. Chaumeil MM, Ozawa T, Park I, Scott K, James CD, Nelson SJ, Ronen SM. Hyperpolarized ¹³C MR spectroscopic imaging can be used to monitor Everolimus treatment in vivo in an orthotopic rodent model of glioblastoma. *NeuroImage.* 2012; 59:193–201. DOI: 10.1016/j.neuroimage.2011.07.034 [PubMed: 21807103]
 248. Park JM, Josan S, Grafendorfer T, Yen Y-F, Hurd RE, Spielman DM, Mayer D. Measuring mitochondrial metabolism in rat brain *in vivo* using MR Spectroscopy of hyperpolarized [2-¹³C]pyruvate: MEASURING MITOCHONDRIAL METABOLISM IN BRAIN USING [2-¹³C]PYRUVATE. *NMR Biomed.* 2013; 26:1197–1203. DOI: 10.1002/nbm.2935 [PubMed: 23553852]
 249. Day SE, Kettunen MI, Cherukuri MK, Mitchell JB, Lizak MJ, Morris HD, Matsumoto S, Koretsky AP, Brindle KM. Detecting response of rat C6 glioma tumors to radiotherapy using hyperpolarized [1-¹³C]pyruvate and ¹³C magnetic resonance spectroscopic imaging. *Magn Reson Med.* 2011; 65:557–563. DOI: 10.1002/mrm.22698 [PubMed: 21264939]
 250. Hurd RE, Yen Y-F, Mayer D, Chen A, Wilson D, Kohler S, Bok R, Vigneron D, Kurhanewicz J, Tropp J, Spielman D, Pfefferbaum A. Metabolic imaging in the anesthetized rat brain using hyperpolarized [1-¹³C] pyruvate and [1-¹³C] ethyl pyruvate. *Magn Reson Med.* 2010; 63:1137–1143. DOI: 10.1002/mrm.22364 [PubMed: 20432284]
 251. Moyer VA. Screening for Prostate Cancer: U.S. Preventive Services Task Force Recommendation Statement. *Ann Intern Med.* 2012; 157:120–134. DOI: 10.7326/0003-4819-157-2-201207170-00459 [PubMed: 22801674]
 252. Kurhanewicz J, Vigneron DB. Advances in MR Spectroscopy of the Prostate. *Magn Reson Imaging Clin N Am.* 2008; 16:697–710. DOI: 10.1016/j.mric.2008.07.005 [PubMed: 18926432]
 253. Tessem M-B, Swanson MG, Keshari KR, Albers MJ, Joun D, Tabatabai ZL, Simko JP, Shinohara K, Nelson SJ, Vigneron DB, Gribbestad IS, Kurhanewicz J. Evaluation of lactate and alanine as metabolic biomarkers of prostate cancer using ¹H HR-MAS spectroscopy of biopsy tissues. *Magn Reson Med.* 2008; 60:510–516. DOI: 10.1002/mrm.21694 [PubMed: 18727052]

254. Hurwitz, AA., Foster, BA., Allison, JP., Greenberg, NM., Kwon, ED. The TRAMP mouse as a model for prostate cancer. In: Coligan AL, John E., editor. *Curr Protoc Immunol*. Vol. Chapter 20. 2001.
255. Lupo JM, Chen AP, Zierhut ML, Bok RA, Cunningham CH, Kurhanewicz J, Vigneron DB, Nelson SJ. Analysis of hyperpolarized dynamic ¹³C lactate imaging in a transgenic mouse model of prostate cancer. *Magn Reson Imaging*. 2010; 28:153–162. DOI: 10.1016/j.mri.2009.07.007 [PubMed: 19695815]
256. Larson PEZ, Bok R, Kerr AB, Lustig M, Hu S, Chen AP, Nelson SJ, Pauly JM, Kurhanewicz J, Vigneron DB. Investigation of tumor hyperpolarized [1- ¹³ C]-pyruvate dynamics using time-resolved multiband RF excitation echo-planar MRSI. *Magn Reson Med*. 2010; 63:582–591. DOI: 10.1002/mrm.22264 [PubMed: 20187172]
257. Keshari KR, Sriram R, Van Criekinge M, Wilson DM, Wang ZJ, Vigneron DB, Peehl DM, Kurhanewicz J. Metabolic Reprogramming and Validation of Hyperpolarized ¹³C Lactate as a Prostate Cancer Biomarker Using a Human Prostate Tissue Slice Culture Bioreactor. *The Prostate*. 2013; 73:1171–1181. DOI: 10.1002/pros.22665 [PubMed: 23532911]
258. Keshari KR, Wilson DM, Chen AP, Bok R, Larson PEZ, Hu S, Criekinge MV, Macdonald JM, Vigneron DB, Kurhanewicz J. Hyperpolarized [2-¹³C]-Fructose: A Hemiketal DNP Substrate for In Vivo Metabolic Imaging. *J Am Chem Soc*. 2009; 131:17591–17596. DOI: 10.1021/ja9049355 [PubMed: 19860409]
259. Kohler, Sj, Yen, Y., Wolber, J., Chen, Ap, Albers, Mj, Bok, R., Zhang, V., Tropp, J., Nelson, S., Vigneron, Db, Kurhanewicz, J., Hurd, Re. In vivo ¹³carbon metabolic imaging at 3T with hyperpolarized ¹³C-1-pyruvate. *Magn Reson Med*. 2007; 58:65–69. DOI: 10.1002/mrm.21253 [PubMed: 17659629]
260. von Morze C, Larson PEZ, Hu S, Keshari K, Wilson DM, Ardenkjaer-Larsen JH, Goga A, Bok R, Kurhanewicz J, Vigneron DB. Imaging of blood flow using hyperpolarized [¹³C]Urea in preclinical cancer models. *J Magn Reson Imaging*. 2011; 33:692–697. DOI: 10.1002/jmri.22484 [PubMed: 21563254]
261. von Morze C, Bok RA, Sands JM, Kurhanewicz J, Vigneron DB. Monitoring urea transport in rat kidney in vivo using hyperpolarized ¹³C magnetic resonance imaging. *AJP Ren Physiol*. 2012; 302:F1658–F1662. DOI: 10.1152/ajprenal.00640.2011
262. Keshari KR, Sriram R, Koelsch BL, Van Criekinge M, Wilson DM, Kurhanewicz J, Wang ZJ. Hyperpolarized ¹³C-Pyruvate Magnetic Resonance Reveals Rapid Lactate Export in Metastatic Renal Cell Carcinomas. *Cancer Res*. 2013; 73:529–538. DOI: 10.1158/0008-5472.CAN-12-3461 [PubMed: 23204238]
263. Sriram R, Van Criekinge M, Hansen A, Wang ZJ, Vigneron DB, Wilson DM, Keshari KR, Kurhanewicz J. Real-time measurement of hyperpolarized lactate production and efflux as a biomarker of tumor aggressiveness in an MR compatible 3D cell culture bioreactor. *NMR Biomed*. 2015; 28:1141–1149. DOI: 10.1002/nbm.3354 [PubMed: 26202449]
264. Clatworthy MR, Kettunen MI, Hu D-E, Mathews RJ, Witney TH, Kennedy BWC, Bohndiek SE, Gallagher FA, Jarvis LB, Smith KGC, Brindle KM. Magnetic resonance imaging with hyperpolarized [1,4-¹³C]fumarate allows detection of early renal acute tubular necrosis. *Proc Natl Acad Sci*. 2012; 109:13374–13379. DOI: 10.1073/pnas.1205539109 [PubMed: 22837393]
265. Laustsen C, Østergaard JA, Lauritzen MH, Nørregaard R, Bowen S, Søgaard LV, Flyvbjerg A, Pedersen M, Ardenkjaer-Larsen JH. Assessment of early diabetic renal changes with hyperpolarized [1-¹³C]pyruvate: Renal Hyperpolarized ¹³C-MRI in Diabetes. *Diabetes Metab Res Rev*. 2013; 29:125–129. DOI: 10.1002/dmrr.2370 [PubMed: 23166087]
266. Laustsen C, Lycke S, Palm F, Østergaard JA, Bibby BM, Nørregaard R, Flyvbjerg A, Pedersen M, Ardenkjaer-Larsen JH. High altitude may alter oxygen availability and renal metabolism in diabetics as measured by hyperpolarized [1-¹³C] pyruvate magnetic resonance imaging. *Kidney Int*. 2014; 86:67–74. [PubMed: 24352155]
267. Lutterbey G, Gieseke J, von Falkenhausen M, Morakkabati N, Schild H. Lung MRI at 3.0 T: a comparison of helical CT and high-field MRI in the detection of diffuse lung disease. *Eur Radiol*. 2004; 15:324–328. DOI: 10.1007/s00330-004-2548-1 [PubMed: 15565313]

268. Brindle KM, Bohndiek SE, Gallagher FA, Kettunen MI. Tumor imaging using hyperpolarized ^{13}C magnetic resonance spectroscopy. *Magn Reson Med*. 2011; 66:505–519. DOI: 10.1002/mrm.22999 [PubMed: 21661043]
269. Thind K, Chen A, Friesen-Waldner L, Ouriadov A, Scholl TJ, Fox M, Wong E, VanDyk J, Hope A, Santyr G. Detection of radiation-induced lung injury using hyperpolarized ^{13}C magnetic resonance spectroscopy and imaging: Detection of RILI. *Magn Reson Med*. 2013; 70:601–609. DOI: 10.1002/mrm.24525 [PubMed: 23074042]
270. Leftin A, Roussel T, Frydman L. Hyperpolarized Functional Magnetic Resonance of Murine Skeletal Muscle Enabled by Multiple Tracer-Paradigm Synchronizations. *PLoS ONE*. 2014; 9:e96399.doi: 10.1371/journal.pone.0096399 [PubMed: 24769864]
271. Bastiaansen JAM, Yoshihara HAI, Takado Y, Gruetter R, Comment A. Hyperpolarized ^{13}C lactate as a substrate for in vivo metabolic studies in skeletal muscle. *Metabolomics*. 2014; 10doi: 10.1007/s11306-014-0630-5
272. DeSantis C, Ma J, Bryan L, Jemal A. Breast cancer statistics, 2013. *CA Cancer J Clin*. 2014; 64:52–62. DOI: 10.3322/caac.21203 [PubMed: 24114568]
273. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin*. 2016; 66:7–30. DOI: 10.3322/caac.21332 [PubMed: 26742998]
274. Lodi A, Woods SM, Ronen SM. Treatment with the MEK inhibitor U0126 induces decreased hyperpolarized pyruvate to lactate conversion in breast, but not prostate, cancer cells. *NMR Biomed*. 2013; 26:299–306. DOI: 10.1002/nbm.2848 [PubMed: 22945392]
275. Brindle KM. Imaging Metabolism with Hyperpolarized ^{13}C -Labeled Cell Substrates. *J Am Chem Soc*. 2015; 137:6418–6427. DOI: 10.1021/jacs.5b03300 [PubMed: 25950268]
276. Ardenkjaer-Larsen JH. On the present and future of dissolution-DNP. *J Magn Reson*. 2016; 264:3–12. DOI: 10.1016/j.jmr.2016.01.015 [PubMed: 26920825]

Figure A.

Signal-time courses of multiple metabolites derived from dynamic HP $[1-^{13}\text{C}]$ pyruvate spectroscopy in a rat. The boxed regions specify the time window for a 17-s CSI acquisition at the following time delays: 15 s, 25 s, and 35 s. The choice of delay determines the best SNR for the metabolites of interest. The insert shows a waterfall plot of the different metabolite peaks: bicarbonate, pyruvate, alanine, pyruvate-hydrate, and lactate (from left to right). Each spectrum was acquired every 3 s. Reproduced from [113].

Figure B. Metabolite maps generated from a single-point 2D CSI pulse sequence, overlaid over a ^1H T_2 -weighted FSE anatomical image of the kidneys. The spectrum (B) is from the $1.25 \times 1.25 \times 1 \text{ cm}^3$ voxel in (A). Reproduced from [104].

Figure C.

A (A) single shot three-dimensional double spin echo pulse sequence and its (B) 3D k-space trajectory. The k-space was acquired as a stack of interleaved spirals. Specific metabolites were excited via a spectral-spatial pulse. Reproduced from [152].

Figure D.

(A) The fate of the ^{13}C labeled carbon-1 (red). In most organs, the label will be detected on the lactate, alanine, bicarbonate (via PDH). In the liver, the label can also appear on other TCA cycle intermediates via the enzyme pyruvate carboxylase (PC). Spectra from (B) fasted and (C) fed states of a perfused, isolated rat liver after injection of HP $[1\text{-}^{13}\text{C}]\text{pyruvate}$. Figures adapted from [178].

Figure E.

The three images are HP $[1-^{13}\text{C}]\text{lactate}/[1-^{13}\text{C}]\text{pyruvate}$ maps overlaid on axial T_2 -weighted ^1H images of the prostate from three patients in the first ever hyperpolarized MR clinical study. All three patients had a biopsy-proven Gleason grade 3 + 3 prostate cancer. Adapted from [103].

Figure F.

(A) The signal-time course of HP lactate after the injection of HP [1- 13 C]pyruvate in isolated, perfused rat lungs of a bleomycin-induced lung injury model. The maximum summed lactate signal is seen on day 7, during the inflammatory phase of this model. The HP lactate signal correlates highly with the semi-quantitative neutrophil score (B), and moderately with the macrophage score (C), based on H&E histology. Adopted from [124].

Figure G.

A multi-bolus functional HP MRS experiment based on an electrical stimulation of hind-limb skeletal muscles in mice. (A) Four boluses of HP [1- ^{13}C]pyruvate were delivered (blue bands), each followed by electrical stimulation (red bands). Each bolus was delivered about 30 s apart; each stimulation was 10 s long and involved 10 ms trains of 200 μs /10 V pulses repeated at 10 Hz. (B) The metabolite-to-total-carbon-ratio (MtoC) of all four boluses with best-fit quantification of each bolus's kinetic data (solid lines). (C) The metabolic rate constants, as determined for each bolus ($p < 0.05$). Reproduced from [270].