Biochemical Specificity in Human Cardiac Imaging by $^{13}$C Magnetic Resonance Imaging

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Energy contained in lactate, glucose, ketones, and fatty acids is captured by metabolic processes in the heart to produce mechanical and electric work. The actual contribution of each substrate to energy production and the specific metabolic pathways involved is sensitive to both physiological conditions and disease. This knowledge of cardiac biochemistry is derived primarily from studies in isolated hearts and from invasive in vivo studies in experimental animals. Animal models of some diseases, notably acute ischemia and reperfusion, provided valuable insights, but in general, the relevance of animal studies to human disease is uncertain because it is difficult to meaningfully model heart failure, hypertrophy, cardiomyopathies, hibernating myocardium, and other complex conditions. Methods to quantify biochemical events in the heart are important because it is becoming increasingly apparent that chronic adaptations in metabolism may drive processes with adverse consequences, such as impaired energy capture and oxidative stress. Positron tomography provides some metabolic information in patients. However, in spite of the popularity and the high sensitivity for detecting a radionuclide, the fit between metabolic complexity and the information accessible by positron tomography is actually poor. For example, uptake, phosphorylation, and trapping of $[13C]FDG$ is widely accepted as a biomarker of glucose metabolism. Yet, the positron tomography signal does not inherently make the simple distinction between metabolism of glucose to acetyl-CoA and subsequent oxidation in the Krebs cycle versus anaerobic glycolysis to pyruvate and lactate. For basic science studies, an alternative to radiotracers is the use of $^{13}$C-enriched substrates with detection by nuclear magnetic resonance (NMR) spectroscopy. $^{13}$C is a stable isotope of carbon that is normally present at $\approx 1\%$ of carbon nuclei. After enriching a compound with $^{13}$C to $\approx 99\%$, intermediary metabolism has been studied for decades using $^{13}$C NMR spectroscopy. $^{1}$ Aside from the convenience of avoiding radiation, $^{13}$C NMR provides high chemical specificity, and it is a simple task to distinguish the many metabolic products and relevant pathways after exposure to $^{13}$C-labeled substrates. However, the sensitivity for $^{13}$C detection in vivo is poor primarily because the concentration of metabolically relevant metabolites is only a few millimolar at best, and the magnetic resonance (MR) sensitivity of $^{13}$C is weak. Consequently, only a few investigators have attempted to detect $^{13}$C in the human heart.

In this issue of Circulation Research, Cunningham et al$^{4}$ report the first images of ventricular myocardium in healthy humans acquired by $^{13}$C MR imaging. This achievement was enabled by a method well known in the physics community, hyperpolarization (HP), to vastly improve sensitivity for detecting $^{13}$C by MR. Dynamic nuclear polarization is based on mixing $^{13}$C-enriched pyruvate with a stable radical, freezing the sample at $\approx 1$ K and exposing the mixture to microwaves (Figure 1A). This results in an increase in $^{13}$C polarization by $10000\times$ or more; hence, the term HP. Many details of dynamic nuclear polarization were well known in the 1970s, but it was not until 2003 that Ardenkjaer-Larsen et al$^{6}$ demonstrated that the frozen sample could be rapidly warmed with boiling water while preserving, albeit temporarily, $^{13}$C polarization. An early application was $^{13}$C imaging of the pig heart. $^{7,8}$ Because of the practical advantages of working with stable isotopes and the potentially high information yield of detecting $^{13}$C with MR methods, there has been intense interest in developing $^{13}$C imaging, particularly for cancer applications. The first results reported in 2013 were obtained in men with prostate cancer. The HP $[1-^{13}C]pyruvate$ was generated in a clean room constructed next to the scanner, and an endorectal coil was used to acquire images.

The current study is somewhat limited by reporting data on only 4 subjects. Nevertheless, the study describes important advances because it confirmed the safety of injecting HP $[1-^{13}C]pyruvate$ in humans. Further, the device used to hyperpolarize pyruvate, the SPINLab, is self-contained and is designed to be located in a clinical environment. HP $[1-^{13}C]pyruvate$ was imaged in the right and left ventricular cavity, and as would be expected from known biochemical pathways (Figure 1B), $[^{13}C]bicarbonate$, $[^{1-13}C]lactate$, $[^{13}C]alanine$, and $^{3}CO_{2}$ were observed.

Relatively homogenous images of HP $[^{13}C]bicarbonate$, derived from HP $[1-^{13}C]pyruvate$, were acquired successfully in the healthy human myocardium. Earlier studies in pigs under general anesthesia were promising, and it is reassuring to see that the experiment worked in humans. $^{7,8}$ The myocardium normally oxidizes primarily fatty acids or ketones, so a transient increase in the concentration of pyruvate might not be sufficient to suppress oxidation of fatty acids and ketones in a
exciting, but what does it mean to detect HP [13C]bicarbonate? Because pyruvate is metabolized in the heart overwhelmingly via pyruvate dehydrogenase, the appearance of HP [13C]bicarbonate in principle serves as a reliable imaging biomarker for flux through this enzyme. Pyruvate dehydrogenase is a large, multienzyme complex that resides exclusively in mitochondria, so presumably the appearance of [13C]bicarbonate indicates intact mitochondria. After prolonged ischemia and reperfusion, the absence of a HP [13C]bicarbonate has been attributed to cardiomyocyte injury. In a model of pacing-induced heart failure, the bicarbonate signal decreased late in the evolution of heart failure. However, in general, the absence of a [13C]bicarbonate signal should be interpreted cautiously because high plasma concentrations of fatty acids or ketones—exactly the situation to be anticipated in sick patients—will suppress oxidation of HP [1-13C]pyruvate even with normally functioning mitochondria. An important challenge going forward is to investigate and validate methods to quantify flux in pyruvate dehydrogenase and the other pathways involved with pyruvate metabolism.

Figure. Schematic of the hyperpolarization process (A) and pyruvate metabolism in the heart (B). [1-13C]pyruvate is mixed with a stable free radical, frozen, and exposed to microwaves to generate hyperpolarized [1-13C]pyruvate. After dissolving with hot water, the solution is rapidly loaded into a syringe for intravenous injection. In the heart, [1-13C]pyruvate may undergo transamination to [1-13C]alanine, reduction to [1-13C]lactate or oxidation to [13C]CO2 and acetyl-CoA (Ac-CoA). Because the activity of carbonic anhydrase is high in the heart, HP[13C]CO2 is rapidly converted to [13C]bicarbonate. TCA indicates tricarboxylic acid cycle.

HP [13C] imaging of the human heart has limitations and disadvantages. The method requires an expensive external device to generate hyperpolarized materials that must be immediately adjacent to the MR scanner. Transfer of the polarized material to the subject must occur within 30 to 50 seconds (Figure 1A). Current [13C] pyruvate, acquisition schemes, and reconstruction algorithms are almost certainly suboptimal. These limitations are primarily technical, and more advanced coils and acquisition schemes will certainly improve image quality. In particular, it is important to assure that signals can be reliably compared from different regions of the myocardium. For example, in Figure 2, there is some variation in HP [13C]bicarbonate signal—does this indicate true differences in metabolism and perfusion of the septum or does it reflect differences in how the coil interacts with tissue in these regions? Once these technical limitations are solved, HP [1-13C]pyruvate will likely provide other insights into metabolism that are simply not available to clinicians today. For example, the [1-13C]lactate signal generated from HP [1-13C]pyruvate occurs largely through an exchange reaction in the active site of lactate dehydrogenase, so it reflects the size of the existing tissue lactate pool, not newly generated lactate from pyruvate. This is important because it offers the potential to image lactate pool sizes in different tissue regions within a few seconds after injection of HP [1-13C] pyruvate. This could potentially allow rapid imaging of tissue ischemia and, in addition, allow monitoring of newly generated lactate after a pharmacological intervention. It must be remembered that the mass of injected [1-13C]pyruvate is significant and potentially could influence cardiac metabolism. Although HP methods provide a new window into cardiac metabolism, it will be important to validate, to the extent possible, with well-accepted existing methods to evaluate metabolism, such as positron tomography and other techniques.

This study demonstrates 2 important points. First, currently available technologies, including the SPINLab as well as radiofrequency coils and pulse sequences, are at least sufficient to begin studies of the myocardium in human subjects. Second, metabolism of [1-13C]pyruvate to downstream metabolites, such as [13C]bicarbonate, can be detected in normal human myocardium, opening the opportunity for better understanding of metabolism in high-impact myocardial disease. Additional 13C-enriched metabolic probes for interrogating other pathways in the heart, such as [1-13C]lactate (see Figure), will likely become available. Because the method inherently provides detailed chemical information about the 13C-enriched probes, it is possible to copolarize 2 compounds, one targeting metabolic processes and the other measuring myocardial perfusion. If the technology can be refined, there are 3 advantages over current methods for a clinician: the absence of ionizing radiation, the capacity to integrate with any other cardiac MR examination, and access to specific information about cardiac metabolism that is not provided by radionuclide methods.

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References


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