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EDITORIAL COMMENT

From Lab to Life

Cardiac Biomarker Measurement in the Intact Heart by Means of Hyperpolarized ^{13}C-Carbon CMR*

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Because troponins leak—as in the case of myocyte necrosis—they are now used as highly sensitive probes to detect myocardial necrosis. Once troponins have leaked from dying myocytes, they diffuse into the bloodstream, and their blood content is then measured in vitro, that is, in the laboratory. In this issue of *iJACC*, Miller et al. (1) demonstrate a novel technique that allows for measuring the leakage of the cardiac biomarker fumarase, directly and noninvasively, in vivo. Importantly, this approach measures enzyme leakage at the site of myocyte damage, locally, in the intact heart. Thus, the presented method translates the laboratory—in vitro—measurement of leaking

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compounds into the in vivo situation, where the activity of leaking enzymes can be measured locally and noninvasively at the level of the organ: in the beating heart. To measure the fumarase activity locally in the myocardium, the authors deliver the substrate—fumarate—by intravenous injection, and this substrate administration is followed immediately by the cardiac magnetic resonance (CMR)-based detection of its conversion into malate, catalyzed by fumarase. This is a very elegant way to measure fumarase leakage, which was proven in both the Langendorff model and in an intact animal model of irreversible myocyte damage induced by ischemia/reperfusion.

HYPERPOLARIZATION TO BOOST THE MAGNETIC RESONANCE SPECTROSCOPY/CMR SIGNAL

To measure leaking compounds in the myocardium, the researchers faced 2 major challenges. First, the test must be highly sensitive to allow the detection of malate production. To address this problem, the researchers used a technique called hyperpolarized CMR. Second, to localize malate production, a novel CMR technique was applied, taking advantage of the extremely high signal offered by hyperpolarized CMR.

Unlike conventional CMR, hyperpolarized CMR starts with the polarization of the magnetic spins to increase magnetization and therefore it increases the signal available for spectroscopy or magnetic resonance (MR)-based imaging. In conventional proton CMR, very few spins—3 to 4 ppm at 1.5-T—are available for signal generation at thermal equilibrium (6 to 8 ppm at 3-T). By hyperpolarization of a compound, for example, of [1,4-$^{13}C_2$]fumarate, 20% to 25% of spins are available for signal generation once this compound is injected into the body. For pyruvate, a precursor of the Krebs cycle, dynamic nuclear polarization can yield polarization of >60% (2). Hence, hyperpolarization can increase the signal by a factor of 10'000 or more (3), and, consequently, biochemistry can be assessed in the living organ in real time (4,5). This technique was then applied to the heart to measure ischemia/reperfusion damage—to measure the accumulation of lactate and reduced bicarbonate production—indicating a halt of the Krebs cycle occurring immediately upon reperfusion (Figure) (6–9). With this technique, it is even possible to measure several molecules produced in the downstream metabolism of the injected hyperpolarized substance. Following the injection of hyperpolarized [1-^{13}C]pyruvate—with the carbon atom hyperpolarized in position-1—the occurrence of [1-^{13}C]lactate, [1-^{13}C]bicarbonate, and [1-^{13}C]alanine during reperfusion can be assessed (8,10), while injection of [2-^{13}C]pyruvate—with the carbon atom hyperpolarized at position-2—the detection of Krebs cycle compounds, such as [1-^{13}C]citrate and [5-^{13}C]glutamate, is feasible, as well as [1-^{13}C]acetylcarnitine, [2-^{13}C]lactate, and [2-^{13}C]alanine (**Figure 1**) (11). With hyperpolarization of ^{13}C-carbon, it is also possible to probe beta-oxidation by injection of free fatty acids such as [1-^{13}C]butyrate

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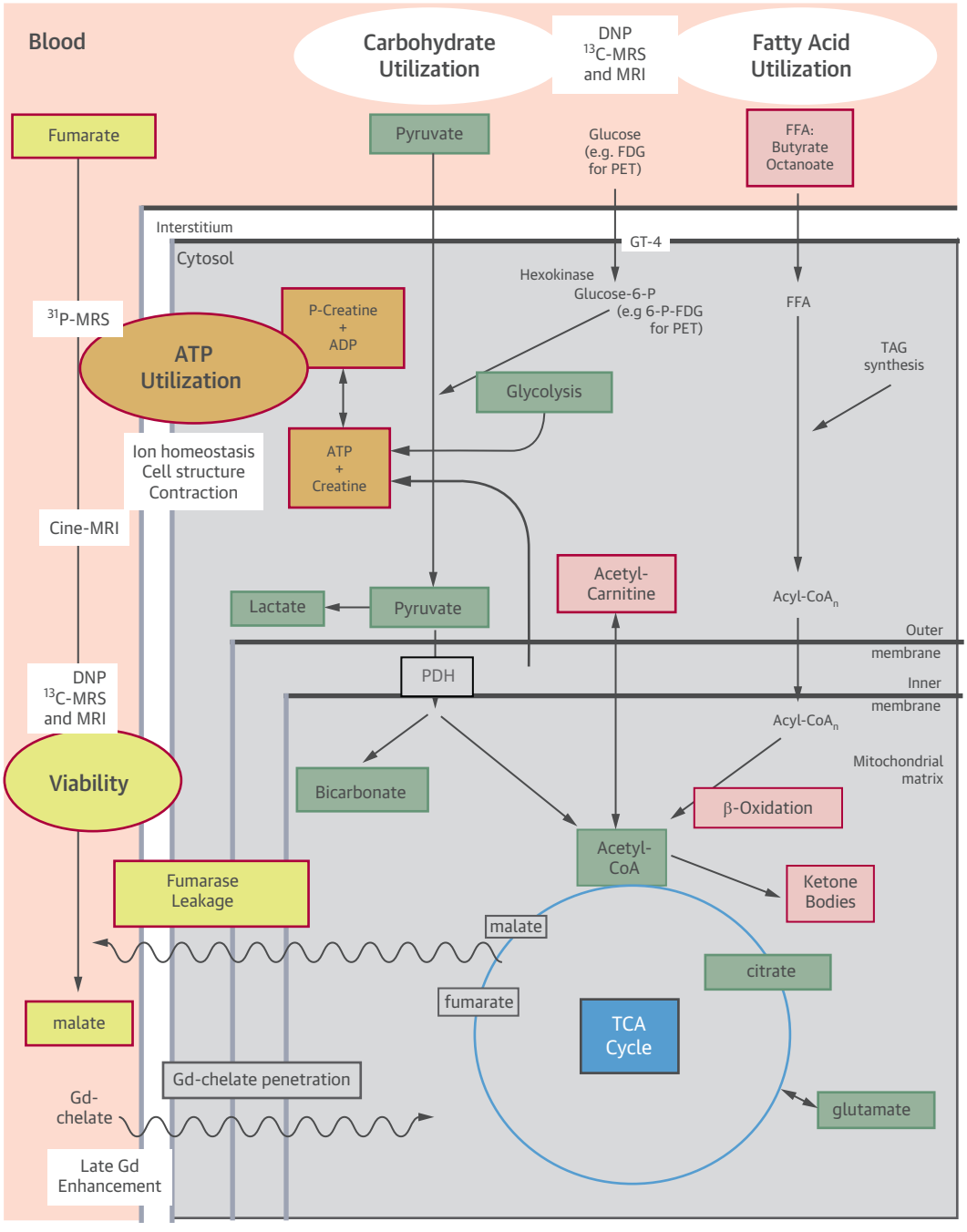
FIGURE 1 Metabolic Processes in the Cardiomyocyte



Dynamic nuclear polarization (DNP) allows probing viability **(yellow boxes)** by detection of malate production. It also allows monitoring carbohydrate **(green boxes)** and fatty acid **(red boxes)** utilization (beta-oxidation pathway). The boxes **(yellow, green, red)** illustrate the downstream compounds that can be detected currently by hyperpolarized ^{13}C-CMR/MRS. For comparison metabolic imaging by positron emission tomography monitors ^{18}F-fluoro-deoxyglucose (FDG) membrane transport (by glucose-transporter-4, GT-4) and its deposition in the cytosol as phosphorylated FDG. In the current study, the authors also performed ^{31}P-MRS to assess ATP and phosphocreatine utilization **(orange boxes)** upon ischemia/reperfusion, and they quantified local contractile response by conventional cine-CMR and strain-CMR. CMR = cardiac magnetic resonance; FFA = free fatty acids; MRS = magnetic resonance spectroscopy; PDH = pyruvate dehydrogenase; TCA = tricarboxylic acid.

or [1-^{13}C]octanoate, and their metabolism can be monitored, ending up in [1-^{13}C]acetyl-carnitine and other compounds (**Figure 1**) (12,13). Even coinjection of different hyperpolarized nontoxic endogenous species is possible, allowing assessment of various metabolic pathways in a single CMR acquisition (**Figure 1**) to investigate, simultaneously, carbohydrate and fatty acid utilization (13).

As hyperpolarization is a short-lived phenomenon, typically lasting 1 to 2 min, only fast biochemical processes can be measured by this technique. However, this feature of short-lived polarization offers a unique possibility to measure the targeted processes repeatedly, such as in the case of progression of leakage, an aspect that cannot be addressed by late gadolinium enhancement, which also probes myocyte membrane integrity (**Figure 1**) (14,15).

IMAGING METABOLISM IN REAL TIME

MR spectroscopy is able to detect this broad spectrum of compounds in near real time. Once the key compounds are identified, a very elegant CMR method (^{13}C chemical shift imaging [CSI]) allows localizing a distinct compound by spatially encoding its signal; with this step, noninvasive imaging of biochemistry in the living organism becomes a reality (16). For probing the pyruvate-dehydrogenase activity, translation into humans by means of CSI following [1-^{13}C]pyruvate injections was successful recently (17).

COMBINING NOVEL TECHNIQUES TO GAIN INSIGHT INTO METABOLIC PROCESSES

To use malate production as a marker of leakage, it is essential to prove that the injected fumarate is not penetrating into the intramitochondrial space. The authors are to be congratulated for their scientific rigor in confirming this assumption by excluding mRNA expression of putative fumarate transporters in heart tissue, which is in line with the lack of malate detection in the healthy heart following injection of fumarate. Also, to relate LDH efflux of the Langendorff heart preparation with quantified malate production is elegant. However, as a limitation of the study, the authors neglected to confirm tissue damage by histology and immunohistochemistry. In their ambitious protocol, Miller et al. (1) not only proved that their technique can detect fumarase leakage, they also acquired both ^{13}Carbon and ^{31}Phosporus MR-spectra to gain insight into the coupling of energy metabolism and necrosis. With these measurements, the onset of necrosis could be related to the phosphocreatine/adenosine triphosphate (ATP) ratio and the myocardial content of ATP. A threshold of approximately 5 mM of ATP was identified (a depletion by ~50%) at which necrosis, i.e., extracellular malate production—was measurable. This part of the study impressively demonstrates how novel techniques can be combined to get metabolic insight in the living intact organism. Thus, the presented approach is a very powerful technical platform to study ischemia/reperfusion in the intact animal, thereby not disturbing the living system under investigation.

With regard to clinical applications, highly sensitive troponin measurements certainly represent the first step in the work-up of symptomatic patients, and, in case of elevated biomarkers and absence of coronary artery obstructions, imaging techniques—such as late gadolinium enhancement and edema imaging—will gain increasing acceptance for identifying the etiology of myocardial tissue damage. Thus, for the next years to come, hyperpolarized ^{13}C-fumarate-based CMR has the potential to propel basic and translational research before it enters the next phase of the clinical arena.

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