Cardiovascular disease is associated with high morbidity, mortality, and financial burden to healthcare services.1–3 In the United States, cardiovascular disease is the leading cause of death in both men and women, accounting for 1 in every 2.9 deaths in 2006, with coronary disease accounting for 1 in every 6 deaths.2 Noninvasive cardiac imaging increasingly plays a fundamental role in diagnosing, assessing prognosis, and monitoring therapy response in cardiovascular disease.4–6 Two-dimensional echocardiography is the most commonly used imaging modality to measure heart function because of its low cost and widespread accessibility. Computed tomography (CT), single photon emission CT, and positron emission tomography (PET) expose patients to ionizing radiation but have been used successfully for clinical assessment of coronary arteries, myocardial perfusion, and viability, respectively. Cardiovascular magnetic resonance (CMR) applies no ionizing radiation and is now considered the gold standard in assessing cardiac anatomy, function, and mass.1 CMR has also shown great potential for evaluating perfusion and viability with gadolinium-based contrast agents.

MR spectroscopy (MRS) and MR-based molecular imaging methods have shown promise for evaluating cardiac metabolism. For example, phosphorus-31 MRS assesses high-energy phosphate content and energy reserve in the human heart (reviewed elsewhere8). Other implementations of multinuclear MRS, including oxygen-17, carbon-13, sodium-23, and proton MRS, have described measurement of oxygen consumption,7 substrate selection, and rates of metabolic flux,8 postinfarct sodium accumulation,9 and lipid accumulation,10 respectively, in ex vivo and in vivo experimental models of disease. MR-based molecular imaging of particles labeled with fluorine-19 nuclei has been used to study tracer and drug pharmacokinetics and metabolism.11 Combined PET–MR imaging (MRI) methods have been demonstrated in preclinical and noncardiac applications to assess cardiac parameters in an infant mouse model12 and for structural, functional, and molecular imaging of patients with brain tumors.13 However, the use of all MR techniques to assess cardiac metabolism in people and to image myocardial perfusion and viability without gadolinium contrast has been limited by an intrinsically low sensitivity.

Hyperpolarization with the dynamic nuclear polarization (DNP) technique can yield >10 000-fold signal increases in MR-active nuclei.14 When used with MRI and/or MRS, hyperpolarized 13C-labeled metabolic tracers allow unprecedented real-time visualization of the biochemical pathways of normal and abnormal metabolism.15 Alternately, the spatial distribution of hyperpolarized 13C-labeled agents can be imaged to achieve high contrast for perfusion and angiographic applications.

In November 2010, hyperpolarized [1-13C]pyruvate was administered to patients for the first time, with a view toward using metabolic MRI to characterize prostate cancer.16 No clinical application in patients with cardiovascular disease has been reported so far; however, given the recent studies in cancer and the experimental application in animal models of cardiovascular disease reported over the past few years, a review of the clinical potential of hyperpolarization techniques in cardiology seems timely. Here, we cover methods for generation of hyperpolarized 13C MR tracers and their experimental use, possible use of hyperpolarized 13C-labeled tracers for human cardiovascular disease, and recent and future technological advances needed for translation of cardiac hyperpolarized 13C MR into the clinic.

Technical Considerations

Principles of Hyperpolarization

The acquisition of images with MRI relies on the fact that the protons in water and fat act like small bar magnets when placed inside a strong magnetic field. They align either with or against the main magnetic field, creating 2 distinct populations. The difference in the size of these 2 populations (known as the polarization) is what determines the strength of the MRI signal (Figure 1). At biological temperatures and field strengths attainable in clinical MR scanners, the difference in these populations is small, leading to a low sensitivity. In clinical MRI, this low sensitivity is compensated for by the high concentration of water and fat in the human body. Unfortunately, this is not the case when other nuclei that are observable with MRI (eg, 13C, 31P, 19F) are used; limited natural abundance and low in vivo concentrations prevent routine clinical monitoring of such nuclei.
A potential way to overcome the limitations of low sensitivity is to use techniques of hyperpolarization that can temporarily align all nuclei in the same direction (Figure 1), increasing the polarization and hence the signal of a particular compound. One such technique, DNP, is of particular interest for metabolic studies because it has the potential to dramatically increase the sensitivity of MRI to molecules containing $^{13}$C nuclei. Hyperpolarization of $^{13}$C-labeled metabolites via DNP relies on the fact that electrons have a very high level of polarization (almost 100%, ie, all electrons aligned in the same direction) when at low temperatures ($<1.4$ K) and in a high magnetic field (typically 3.35 T). The DNP process, shown in Figure 1, transfers the electronic polarization to the $^{13}$C-labeled molecule of interest through the use of microwave irradiation. This is achieved by mixing a source of free electrons (called a radical) with the $^{13}$C-labeled sample to be hyperpolarized. The mixed sample is then placed in a high magnetic field and rapidly frozen in liquid helium. The temperature is reduced further by vacuum pumping the sample to very low pressures ($<1$ mbar) to bring the final thermal equilibrium polarization and MR signal.

![Figure 1. A](image)

**A.** At thermal equilibrium, when magnetic resonance (MR)-active nuclei are placed in a magnetic field, spins that align parallel to the magnet field have a slightly lower energy than spins that align antiparallel to it. The energy advantage obtained by aligning parallel to the magnetic field causes slightly more spins to point in that direction; ie, it causes a net polarization, which results in the production of MR signal. However, thermal equilibrium polarization is extremely low (on the order of only 0.0005%), and this translates directly into low MR signals. **B.** By forcing most spins to point in the same direction, dynamic nuclear polarization (DNP) achieves polarization levels of $>20\%$, which translates into an equivalent 10 000-fold increase in MR signal. **C.** To achieve DNP, MR-active nuclei such as $^{13}$C are mixed with a low concentration of free electrons, and the sample is irradiated with microwaves in a high magnetic field ($\approx 3$ T) and at low temperatures ($\approx 1$ K). The hyperpolarizer system (left) also allows sample dissolution to temporarily maintain the high signal in solutions with a physiological temperature and pH for injection.

![Figure 2](image)

**Figure 2.** The metabolic pathways that have been studied with hyperpolarized $^{13}$C-labeled metabolic tracers and have the potential to be diagnostic targets for cardiovascular disease. LDH indicates lactate dehydrogenase; AAT, alanine aminotransferase; CA, carboxic anhydrase; CAT, carnitine acetyltransferase; GDH, glutamate dehydrogenase; and PDH, pyruvate dehydrogenase.
temperature to approximately 1 K. Microwave irradiation, at a frequency determined by the properties of the $^{13}$C molecule, the radical, and the magnetic field strength, is then applied to transfer the polarization from the electrons to the carbon molecules. Depending on the molecule to be polarized, this process typically takes between 30 and 60 minutes and results in a $^{13}$C polarization of up to 50% (ie, twice as many nuclei pointing in 1 direction). Such polarization levels are much greater than the normal MRI polarization levels of $\approx 0.0005\%$

Obviously, the biological applications of a molecule at 1 K are limited; therefore, it is necessary to bring the sample to a physiological temperature before use in an in vivo experiment. To achieve this, the hyperpolarized sample is rapidly dissolved by the injection of a heated and pressurized bolus of aqueous solvent. The resulting solution retains a high level of nuclear polarization ($\approx 20\%$ to 40%) and can be formulated to be at physiological temperature and pH for in vivo injection. The polarization produced then steadily decays back to a normal level (thermal equilibrium) at a rate dependent on the inherent properties of the molecule under study (typically 1–2 minutes). Thus, a current limitation of the method is that the enhanced signal is available for only a short period of time.

Theoretically, the DNP technique can be applied to a wide range of molecules labeled with $^{13}$C or any other MR-active nucleus. However, for metabolic imaging, several criteria must be met for the successful polarization and in vivo detection of the hyperpolarized molecule and its products. This includes limitations on the molecular properties and the speed of uptake and use (for a full review, see the work by Gallagher et al[17]). Hyperpolarized $^{13}$C MR studies have used the molecules [$1^{-13}$C]pyruvate, [$2^{-13}$C]pyruvate, and $^{13}$C-bicarbonate most frequently because they polarize efficiently, retain hyperpolarization for a relatively long time (time constants of signal decay, $\approx 50$ seconds), and are rapidly metabolized by tissues to provide flux measurements through important enzymes in the heart, as shown in Figure 2.

Requirements for Clinical Translation and Application

In this review, we summarize work that has used the DNP dissolution process, coupled with $^{13}$C MR, to further our understanding of cardiovascular disease. We also speculate on the clinical potential of the technology, highlighting the areas of cardiovascular medicine in which its application appears promising. Rapid advances have been made since the advent of the dissolution DNP process, in terms of both technological understanding and biological application, which means that the technique is used by an increasingly large number of preclinical research groups, with particular emphasis on oncology and cardiovascular medicine.

The true potential of hyperpolarized $^{13}$C MR lies in its translation to clinical research and clinical diagnosis and management. Toward this goal, the “first trial in human” studies were carried out in November 2010 at the University of California at San Francisco using hyperpolarized [$1^{-13}$C]pyruvate in the staging of prostate tumors. Metabolic signatures appropriate to benign and cancerous tissue were seen, with elevated lactate levels within the tumor. Achieving the goal of routine clinical application will require considerable technological advances in terms of improved methods and hardware for the acquisition of $^{13}$C images and access to affordable hyperpolarization tools and $^{13}$C-labeled compounds. Furthermore, the widespread use of hyperpolarized $^{13}$C MR methods in the clinic will require that the technique provides a clear advantage over other noninvasive diagnostic technologies in improving clinical outcome.

Hyperpolarized $^{13}$C MR offers many theoretical advantages over existing metabolic imaging techniques (eg, PET). First, the method does not use ionizing radiation, making the procedure an ideal candidate to satisfy the current demand for cardiovascular imaging strategies that minimize patient radiation exposure. Second, the inherent ability of MRI to encode both spatial and spectral information means that it is possible to distinguish between the injected tracer and its downstream metabolic products. This enables metabolic pathways to be tracked over multiple enzyme-regulated steps, potentially yielding sensitive and specific indicators of disease. Third, hyperpolarized $^{13}$C-labeled tracers are normal physiologically occurring compounds, minimizing the risk of adverse effects from pharmacological interactions and potentially providing an alternative to contrast agents that are contraindicated in certain patient groups (eg, gadolinium-based agents in patients with advanced kidney disease). Finally, the potentially high cost of hyperpolarized $^{13}$C metabolic tests can be minimized by incorporating the hardware into existing MR facilities and the rapid $^{13}$C MRI and MRS scans into standard CMR protocols. Such incorporation should be relatively trivial because the additional hardware demands (multinuclear transmit/receive channels and RF coils) are simple additions to existing MRI equipment.

All imaging techniques have their specific disadvantages, and hyperpolarized $^{13}$C MR is no exception. The greatest challenge presented by the technique is the rapid decay of MR signal after DNP and dissolution, which means that a hyperpolarized tracer must be injected in vivo as soon after it is produced as possible. Signal decay restricts the compounds that can be hyperpolarized; their relaxation must be slow enough to ensure that they can be administered in vivo and, once administered, they must be metabolized quickly. Additionally, to minimize tracer delivery time, the polarizer equipment must be sited immediately beside the MR system, which introduces the need for sterile handling of compounds within the MRI suite and the associated regulatory issues. This issue has been addressed to some extent by the design of a sterile polarizer[20] and the granting of an Investigational New Drug approval for the use of hyperpolarized [$1^{-13}$C]pyruvate by the Food and Drug Administration. However, the regulatory issues surrounding the widespread use of new hyperpolarized compounds remain a hurdle to be addressed.

A second limitation of the technique is that, despite the large gains in sensitivity over thermal equilibrium MRI, the technique does not reach the sensitivity of PET, which can detect extremely low concentrations of radioactive tracer in the nanomolar range, albeit with a low spatial resolution. Therefore, acquisition of sufficient MR signal requires the injection of metabolic tracers at doses in the micromolar to
millimolar range. This disadvantage is partially ameliorated by the fact that the infused compounds are naturally occurring. However, metabolic tracer levels that approach or exceed physiological levels of the unlabeled compound will be required and their safety in humans will need to be shown. Thus, the metabolic effects of the injection itself will need to be well understood in the interpretation of the results.

Applications in the Study of Cardiovascular Disease and Clinical Potential

Normal Heart

Metabolic Substrate Use in the Normal Heart

To meet its task of continually circulating blood throughout the body, the heart consumes more energy in the form of ATP than any other organ. The healthy heart derives 60% to 90% of its energy from the oxidation of fatty acids, with the remainder primarily from pyruvate oxidation, derived from glucose (via glycolysis) and lactate. However, when plasma substrate composition is altered, the relative contributions of lipids, carbohydrates, and ketone bodies to cardiac ATP production vary substantially. Several PDH-mediated mechanisms exist to promote fatty acid oxidation over glucose oxidation, including phosphorylation-inhibition of PDH and end-product inhibition of PDH by acetyl-CoA and NADH. However, metabolic flexibility remains the key characteristic of the heart to meet high ATP demand. After eating and in response to energetic stressors such as aerobic exercise or ischemia, glucose and other carbohydrates become more predominant cardiac fuels.

Hyperpolarized $^{13}$C MRS to Investigate Normal Cardiac Metabolism

Initially, hyperpolarized $^{13}$C MRS measurements of in vivo substrate selection were validated via comparison with analogous data collected in vitro and ex vivo. It was first demonstrated in the isolated perfused rat heart that infusion of hyperpolarized [1-$^{13}$C]pyruvate and MRS detection of total carbonic acid ($^{13}$CO$_2$ plus $^{13}$C-bicarbonate) measured flux through the PDH enzyme complex. The operation of the glucose–fatty acid cycle in vivo was subsequently demonstrated in a study using hyperpolarized [1-$^{13}$C]pyruvate and MRS to measure PDH flux in fed and fasted rats (Figure 3A). As Randle et al observed in vitro in 1963, elevated plasma free fatty acids, typical of the fasted state, reduced PDH-mediated glucose oxidation in vivo compared with the fed state. The direct correlation between PDH flux measured in vivo with hyperpolarized [1-$^{13}$C]pyruvate and PDH activity measured with an in vitro assay has been demonstrated in rats in which PDH activity was increased from low to high rates (0.77–6.75 mol·min·g·wet weight) with metabolic interventions including high-fat feeding and pharmacological PDH activation with dichloroacetate. Thus, the study of PDH activity and its control has been a major application of hyperpolarized [1-$^{13}$C]pyruvate.

Real-time Krebs cycle metabolism has also been followed in the heart with $^{13}$C MRS by shifting the $^{13}$C label to the second carbon of pyruvate. When hyperpolarized [2-$^{13}$C]pyruvate is used as a metabolic tracer, the $^{13}$C label is retained within acetyl-CoA rather than being released as $^{13}$CO$_2$, enabling downstream metabolic steps to be visualized (Figure 4). The enzymatic conversions of hyperpolarized...
[2-13C]pyruvate to [2-13C]lactate, [1-13C]acetyl-carnitine, [1-13C]citrate, and [5-13C]glutamate were observed with subsecond temporal resolution in the perfused and in vivo rat heart.34,35 The appearance of 13C in the glutamate pool was delayed by 3 seconds compared with citrate in vivo, demonstrating the feasibility of measuring instantaneous flux through the first span of the Krebs cycle and the oxoglutarate-malate carrier.

Rapid mitochondrial cycling between acetyl-CoA and an acetyl-carnitine substrate buffer was revealed by following real-time hyperpolarized [2-13C]pyruvate metabolism.35 Understanding the role of acetylcarnitine in "fine-tuning" mitochondrial acetyl-CoA supply is important in the healthy heart because carnitine deficiency has been reported in pathophysiological conditions in which cardiac energetics are also depleted, including old age,6 pressure-overload hypertrophy,17 and heart failure.38,39 Further advances in our understanding of cardiac metabolism will undoubtedly be forthcoming as, for the first time, hyperpolarized 13C MR enables in vivo metabolic processes to be monitored, implying that results will be obtained with preserved myocyte structure and environment, physiologically high cardiac workload, and maintenance of the plasma substrate and neurohumoral composition (with the exception of minor disturbances caused by the metabolic tracer itself).

In the future, when hyperpolarized 13C MR methods are approved for cardiovascular use in humans, studies of metabolism in the healthy heart will be needed. These studies will be vital in optimizing protocols for cardiac hyperpolarized 13C MRI and MRS in terms of prescan preparation to establish a reproducible metabolic state, identification of tracer doses required, and refinement of MR data acquisition and data processing methods. In addition, defining metabolic substrate selection noninvasively in the healthy human heart will be fundamental to the development of drugs targeting cardiac metabolism. In particular, showing that the glucose–fatty acid cycle operates in humans is of great importance; excess free fatty acid oxidation at the expense of glucose oxidation may adversely affect the heart in a variety of conditions caused by raised plasma free fatty acids levels, causing the insulin resistance found in diabetes mellitus, the metabolic syndrome, obesity, and chronic heart failure.6,40,41

Ischemic Heart Disease

Preliminary evidence indicates that hyperpolarized 13C MRI could have a role in diagnosing ischemic heart disease by directly detecting the metabolic consequences of myocardial ischemia, assessing the coronary arteries, mapping myocardial perfusion, and demonstrating myocardial viability.

Understanding Acute Ischemia/Reperfusion Injury

The metabolic consequences of ischemia have been characterized extensively in experimental animal models. In ischemia, primary metabolic adaptations include increased reliance on glycogen breakdown and glucose uptake as a source of energetic fuel42 and reliance on anaerobic glycolysis for energy production,43 with decreased Krebs cycle metabolism and oxidative phosphorylation resulting from low oxygen availability.44 The end products of glycolysis include protons (with an associated acidosis), lactate, and NADH, which increases the reduction-oxidation (redox) potential of affected cardiomyocytes.42,43,45–47

Experiments performed in the globally ischemic, isolated rat heart have demonstrated that hyperpolarized 13C-pyruvate can show the glycolytic switch characteristic of myocardial ischemia. In hearts perfused with Krebs-Henseleit buffer and either pyruvate alone48 or glucose plus pyruvate,34,49 total global ischemia for 10 minutes resulted in substantially increased [1-13C]citrate and [5-13C]glutamate pools.34

Hyperpolarized tracers also enable noninvasive measurement of intracellular and extracellular pH (pHi and pHe).
After the infusion of hyperpolarized $^{13}$C-bicarbonate intravenously in mice with subcutaneously implanted tumors, MR imaging showed the distribution of hyperpolarized $^{13}$C-bicarbonate and $^{13}$CO$_2$. A pH map was then generated throughout the tumor with the ratio of $^{13}$C-bicarbonate to $^{13}$CO$_2$ and the Henderson-Hasselbalch equation (Figure 5). In tumors, this approach succeeded because the high carbonic anhydrase activity on the surface of tumor cells and within erythrocytes ensured virtually instantaneous equilibrium between the infused $^{13}$C-bicarbonate and $^{13}$CO$_2$. In the heart, use of hyperpolarized $^{13}$C-bicarbonate would also be expected to accurately measure pH in vivo because the red cell carbonic anhydrase activity is maintained. This may become a sensitive imaging marker of ischemia.

Hyperpolarized $^{13}$CO$_2$ and $^{13}$C-bicarbonate generated as a byproduct of [1-$^{13}$C]pyruvate metabolism can measure in vivo cardiac pH. Isolated perfused heart studies, in vivo studies, and mathematical modeling established that $^{13}$CO$_2$ produced by mitochondrial PDH remains within cardiomyocytes for a sufficiently long time to equilibrate with $^{13}$C-bicarbonate. Therefore, detection of the ratio of cardiac $^{13}$C-bicarbonate to $^{13}$CO$_2$ after hyperpolarized [1-$^{13}$C]pyruvate infusion offers a novel noninvasive technique to measure cardiac pH. Magnetization-transfer $^{31}$P MRS and amide proton transfer imaging have previously been used to measure pH in vivo; however, low sensitivity and limited field homogeneity resulting from cardiac motion, respectively, imply that hyperpolarized [1-$^{13}$C]pyruvate may be the first method for mapping cardiac pH that could realistically be applied in patients.

In coronary artery disease patients, myocardial ischemia is a regional phenomenon with variable transmural severity, being more severe in the endocardium than in the epicardium and ranging in severity from transient and reversible (angina, stunning, hibernation) to prolonged and irreversible (myocardial necrosis and scar). In between these 2 extremes, many clinical situations can be recognized in which a mixture of viable and necrotic/scarred tissue exists. Characterizing the metabolic patterns unique to ischemic myocardium in coronary artery disease patients has been complicated by tissue heterogeneity and the difficulty of making non–steady-state measurements of lactate production and/or pH from the precise site of the ischemic zone. Therefore, using hyperpolarized $^{13}$C MRI to image localized changes in lactate accumulation and pH noninvasively and with high spatial resolution may be valuable in clinical research to reveal the metabolic changes occurring with acute ischemia/reperfusion injury in humans. Using hyperpolarized $^{13}$C MRI to show ischemia may have a direct clinical application in determining the immediate tissue outcome of procedures such as percutaneous coronary intervention or coronary artery bypass graft. MR imaging of hyperpolarized [1-$^{13}$C]pyruvate metabolism that shows normal pH ($7.0–7.2$) and minimal $^{13}$C-lactate production may indicate that blood flow is adequately restored. Furthermore, hyperpolarized $^{13}$C MRI examination immediately after primary percutaneous coronary intervention may indicate a patient’s potential for recovery and degree of irreversible damage. Hyperpolarized $^{13}$C MRI may also contribute to clarifying the role of ischemia in cardiac syndrome X.

**Demonstrating Coronary Anatomy: Coronary Angiography**

Despite the associated doses of ionizing radiation, cardiac CT is the current gold standard for the noninvasive assessment of the coronary arteries based on calcium scoring and CT coronary angiography. Although progress has been made, reliable coronary lumen imaging with CMR has been hindered by the high temporal and spatial resolution required to image stenosed arteries accurately. However, when hyperpolarized $^{13}$C-labeled substrates are imaged with MRI, the
lack of background signal means that acquired images show only structures containing the infused contrast agent. The resultant gains in the signal-to-noise and contrast-to-noise ratios may enable rapid, high-resolution imaging of the coronary arteries with MRI, which would allow direct and repeatable assessment of coronary arteries without the use of ionizing radiation.

Toward this goal, Golman and Petersson provided the first evidence that hyperpolarized $^{13}$C MRI, with either dissolution DNP methods or other hyperpolarization techniques, may be useful to visualize the coronary arteries. Hyperpolarized $^{13}$C-urea was infused via a catheter after selective intubation of the left coronary artery in healthy pigs, and the coronary arteries were imaged with a balanced steady-state free-progression MRI acquisition sequence. As an initial proof-of-concept study, the results were promising. However, the images were acquired with a projection imaging technique (slice thickness, 15 cm), and the in-plane spatial resolution ($\approx 2 \times 2$ mm) was an order of magnitude away from that achievable with CT. The temporal resolution of the images was 422 milliseconds (1 image acquired every heartbeat), still some way from that achievable with CT ($\approx 80$ milliseconds).

The limited spatial resolution currently achievable with hyperpolarized $^{13}$C MRI is the result of both the short acquisition time (caused by the decay of the enhanced signal) and the demands that $^{13}$C imaging places on the gradient system of the MRI system owing to the low gyromagnetic ratio of $^{13}$C (4 times lower than $^1$H). Future hardware improvements may help to overcome the gradient limitations, and the development of new intravascular hyperpolarized agents with a longer relaxation time, possibly based on other nuclei (eg, $^{15}$N), may improve the achievable spatial resolution. However, the need to limit background signal may require direct infusion of the tracer into the coronary arteries. This would still necessitate invasive catheterization, limiting the advantage of hyperpolarized $^{13}$C MRI over conventional x-ray coronary angiography to the elimination of the radiation burden.

**Demonstrating Stress-Inducible Ischemia**

In the wake of the Clinical Outcomes Utilizing Revascularization and Aggressive Drug Evaluation (COURAGE) trial, increased importance has been placed on demonstrating inducible myocardial ischemia before initiating an elective coronary intervention. MRI of myocardial perfusion with a gadolinium-based contrast agent during pharmacological stress has emerged as a highly sensitive and specific technique for noninvasive demonstration of reversible, stress-inducible ischemia. MRI has a number of advantages over other established techniques such as single photon emission CT and stress echocardiography, including high spatial and temporal resolution, no exposure to ionizing radiation, no attenuation or scatter artifacts, and no image orientation constraints. However, quantitative analysis of MR perfusion images remains time consuming, and most centers continue to limit analysis to visual assessment. This is usually sufficient to identify perfusion defects, although quantitative image analysis may be needed to identify stress-inducible ischemia in patients with 3-vessel disease and balanced myocardial hypoperfusion.

Unlike gadolinium-based contrast agents, hyperpolarized agents directly generate MR signal, so $^{13}$C MR signal intensity is proportional to tissue $^{13}$C concentration and therefore tissue perfusion. Imaging the distribution of a nonmetabolized hyperpolarized agent is a theoretically simple method for quantitative perfusion mapping to demonstrate stress-inducible ischemia. Golman and Petersson have demonstrated the feasibility of imaging myocardial $^{13}$C distribution to map perfusion. Hyperpolarized $^{13}$C-urea was infused intravenously into a pig, and images were acquired with a balanced steady-state free-progression pulse sequence and $3 \times 3$-mm in-plane spatial resolution with 10-mm slice thickness. On occlusion of the left anterior descending artery, no signal was measured in the corresponding coronary territory. Furthermore, the authors calculated quantitative myocardial perfusion maps on a pixel-by-pixel basis using the Key-Schmidt method after correcting for hyperpolarized tracer signal decay.

To extend hyperpolarized $^{13}$C MRI methods of quantitative perfusion imaging to patients, hyperpolarized agents that are not metabolized in vivo should be developed that have long relaxation times, and their ability to indicate myocardial perfusion should be evaluated under rest and adenosine stress conditions. Furthermore, images illustrating both the perfusion and metabolism of hyperpolarized [1-13C]pyruvate at rest and during adenosine stress should be compared. In stress-inducible ischemia, it may be possible to detect regional increases in the conversion of [1-13C]pyruvate into [1-13C]lactate, which could indicate perfusion defects in individual coronary territories.

Incorporation of hyperpolarized agents into conventional MR myocardial perfusion imaging methods could generate a technique that is, conceptually, much like quantitative single photon emission CT but without the use of ionizing radiation. Furthermore, the spatial resolution achieved by Golman and Petersson (3-mm in-plane spatial resolution, 10-mm slice thickness) is already superior to that attainable with single photon emission CT, and the future development of novel pulse sequences for $^{13}$C perfusion mapping will undoubtedly improve this resolution.

Although hyperpolarized $^{13}$C-labeled agents may simplify quantification relative to conventional MR-based perfusion methods, their use in the clinic would increase imaging complexity and cost, and it is currently unclear whether the spatial and temporal resolution of hyperpolarized $^{13}$C MRI will be able to compete with that of gadolinium contrast agent–based perfusion methods. Potential advantages for the use of hyperpolarized $^{13}$C MRI over gadolinium-based approaches may be the demonstration of stress-inducible ischemia in patients with suspected 3-vessel disease and balanced hypoperfusion, who require quantitative perfusion imaging (which should be more straightforward for hyperpolarized approaches), and the use in patients with advanced kidney failure, who would not be suitable for gadolinium-based contrast agents.
Demonstrating Myocardial Viability
In patients with ischemic heart disease, surgical revascularization of akinetic, yet viable, tissue significantly improves outcome.66–70 The use of noninvasive imaging to identify regions of stunned and hibernating versus necrotic and scarred myocardium (ie, the determination of tissue viability) is essential for the optimal treatment of patients with ischemic heart disease and reduced left ventricular function considered for revascularization.66,67,70–72 Currently, the most promising techniques to assess myocardial viability include CMR with late gadolinium enhancement (LGE) and PET perfusion/metabolism mismatch studies performed with the tracers 18F-FDG and 13N-ammonia.73 In CMR with LGE, viability can be quantified by the transmurality of hyperenhancement in dyskinetic or akinetic segments,1,69 and the transmural extent of scar correlates closely with the likelihood of segmental recovery.69,74

A study by Golman and colleagues used CMR with LGE as a gold standard to verify that hyperpolarized 13C MR could demonstrate myocardial viability.51,75 Hyperpolarized [1-13C]pyruvate was infused intravenously into a pig after either a 15- or 45-minute occlusion of the left circumflex and 2 hours of reperfusion. 13C metabolic images were acquired with an in-plane spatial resolution of 5×7.5 mm, a slice thickness of 20 mm, and a total acquisition time of 13.4 seconds. Metabolic images were compared with CMR-LGE images acquired 15 minutes after gadolinium infusion, with 3×3-mm in-plane spatial resolution and a slice thickness of 10 mm. After the 15-minute occlusion, CMR revealed hypokinetic wall motion with no accompanying hyperenhancement on LGE, indicative of stunned myocardium. The accompanying metabolite maps revealed suppressed PDH-mediated 13C-bicarbonate production that was colocalized with the hypokinetic areas (Figure 6). After a 45-minute occlusion, hyperenhancement indicating irreversible damage was detected, and production of both 13C-bicarbonate and [1-13C]alanine was reduced in the corresponding area (Figure 6). This suggests that not only can hyperpolarized 13C distinguish between viable and necrotic myocardium, as can LGE, but, unlike LGE, it also can distinguish between normal and akinetic viable myocardium because of the unique metabolic signatures of each condition.

One major advantage of hyperpolarized methods over CMR-LGE and PET may be the time efficiency of the method. In the study by Golman and colleagues,51,75 hyperpolarized 13C MRI required only 1 bolus of contrast agent and a 13.4-second scan time to distinguish between viable and nonviable tissue. Clinically, this would provide a logistic benefit over CMR-LGE, which requires scan times of 5 to 20 minutes, and PET, which requires a dual bolus of radioactive tracer and scan times approaching 1 hour.76

Future development of the technique is necessary to determine whether hyperpolarized 13C MRI can assess myocardial viability at relevant doses of infused [1-13C]pyruvate and whether the technique will bring the spatial resolution required to assess tissue in heterogeneous ischemic zones and to determine the transmural extent of scar. Although the spatial resolution of hyperpolarized 13C MRI compares favorably with that of PET, it remains to be seen whether improvements to 13C data acquisition methods can achieve spatial resolution that approaches the resolution attainable with CMR-LGE.

Heart Failure
Metabolic Substrate Use in the Failing Heart
The occurrence of metabolic dysfunction in heart failure is undisputed, yet the causal roles of altered substrate use and myocardial energetics remain controversial.6,23 Patient studies performed with 31P MRS and PET imaging have confirmed that cardiac energetics are abnormal throughout the progression of heart failure. Myocardial phosphocreatine/ATP ratios, as determined with 31P MRS, are reduced in heart failure, correlate with New York Heart Association classes and with indexes of systolic and diastolic function, and predict prognosis.79 PET studies have demonstrated that, throughout the progression of heart failure, the balance between glucose and fatty acid oxidation shifts80–82 and that many treatments, including β-blockade and cardiac resynchronization therapy, normalize myocardial oxidative metabolism and efficiency.81,83,84 However, controversy remains over the exact nature of these metabolic alterations,85 with the relative use of fatty acids increased, maintained, or decreased, depending on the origin and stage of disease.5,23,86 Changes in glucose use in the failing heart are also poorly understood.9 This controversy is due in part to the fact that myocardial metabolic investigations are carried out with either destructive in vitro methods or in vivo radiolabeled tracer techniques, including PET. PET studies in patients have yielded inconsistent results describing the nature of shifts in substrate selection that occur with heart failure, depending on the disease origin, patient preparation before the study, and whether the PET tracers were oxidized (11C-glucose and 13C-palmitate) or measured only substrate uptake (18F-FDG and 13N-fluoro-6-thiaheptadecanoic acid81,82). To understand the timing and consequences of switches in substrate metabolism during the development of heart failure and to potentially use those switches for disease diagnosis, a noninvasive method is required that is capable of serially monitoring in vivo cardiac metabolism.85

Hyperpolarized 13C MR to Investigate Metabolism in the Failing Heart
Studies are currently using hyperpolarized 13C MR to examine substrate selection throughout the progression of heart failure, in a rat model of left ventricular remodeling after myocardial infarction,87 and in a porcine tachycardia-induced model of dilated cardiomyopathy.88 These studies have serially examined hyperpolarized [1-13C] and [2-13C]pyruvate metabolism with MRS and/or MRI and have demonstrated reduced incorporation of the 13C label into the [5-13C]glutamate pool throughout the progression of the disease.

In the future, if serial 13C measurements are correlated with cine MRI measurements of cardiac structure and function,31P MRS measurements of cardiac energetics, and biochemistry and histology, it may be possible for these studies to gain unique insight into the temporal relationships that exist between alterations in substrate metabolism, energetics, and function and to determine whether altered metabolism is a
cause or consequence of heart failure. Distinct profiles of 
13C-labeled tracer metabolism may emerge that specifically 
correlate with cardiomyopathy stage, genotype, and origin. If 
this is the case, metabolic profiling with hyperpolarized 13C 
MR may become a useful technique for cardiologists to 
predict heart failure patient prognosis and ability to recover 
function and to optimize disease management on the basis of 
stage and origin. For example, determination of 13C 
metabolic profiles may help to diagnose hypertrophic cardio-
myopathy in cases of borderline or overlapping phenotypes. 
Application of hyperpolarized 13C MR to the failing heart 
may also aid the design and evaluation of novel treatments. 
New drugs for normalization of cardiac metabolism and 
energetics may emerge on the basis of specific metabolic 
defects identified by hyperpolarized 13C MR. Serial 13C 
metabolic profiling may also be useful to monitor the 
efficacy of new heart failure treatments, particularly met-
abolic modulators such as trimetazidine and carnitine 
palmitoyltransferase-1 inhibitors, but also nonmetabol-
ic drugs and interventional therapies that may improve 
cardiac efficiency. Finally, metabolic profiling with 
hyperpolarized 13C MR may be used to monitor the 
efficacy of new heart failure treatments, particularly met-
abolic modulators such as trimetazidine90,91 and carnitine 
palmitoyltransferase-1 inhibitors, but also nonmetabol-
ic drugs and interventional therapies that may improve 
cardiac efficiency.92,93 A consensus has yet to be reached regarding the precise diagnostic criteria.

Other Forms of Metabolic Heart Disease

Diabetic Cardiomyopathy
Diabetic cardiomyopathy is defined as the alteration of 
cardiac structure and function induced independently by 
diabetes mellitus in the absence of ischemic heart disease, 
hypertension, or other cardiac pathologies. A consensus 
has yet to be reached regarding the precise diagnostic criteria.

Figure 6. Proton images, semiquantitative gadolinium perfusion maps, and late gadolinium enhancement (LGE) images, together with 
[1-13C]alanine and 13C-bicarbonate metabolic maps, obtained in a pig after a 15-minute and a 45-minute occlusion of the left circum-
flex artery. The perfusion and metabolic maps have been superimposed on the proton image. These images indicate the potential of 
hyperpolarized 13C magnetic resonance imaging to demonstrate myocardial viability in patients with ischemic heart disease. After 15 
minutes of occlusion, the combination of preserved [1-13C]alanine production with a regional defect in 13C-bicarbonate production con-
fi rms the presence of “stunned ” but viable myocardium (as determined by assessment of wall motion with proton cine magnetic res-
onance imaging). After 45 minutes of occlusion, defects in both the [1-13C]alanine and 13C-bicarbonate metabolic maps correlated with 
a region of nonviable myocardium, as indicated by perfusion imaging and LGE. Reprinted from Golman et al75 with permission of the 
publisher. Copyright © 2008, John Wiley and Sons Inc.

Figure 7. In vivo data acquired with a multislice cardiac-gated sequence showing spatial distribution of metabolites in a short-axis view 
of the healthy pig heart. Bicarbonate, lactate, and pyruvate volumes were acquired over 9 heartbeats, and the sequence was repeated 
for 3 time points. Data were acquired with a surface coil, resulting in higher signal detection from the anterior wall of the myocardium 
compared with the posterior wall. The resolution of the overlaid reconstructed images is 8.8×8.8 mm² in plane with a 1-cm slice thick-
ness. The entire scan was completed within 18 seconds. The data acquired with this novel pulse sequence illustrate the increasingly 
high spatial and temporal resolution with which cardiac 13C metabolic images can be acquired. Furthermore, images of pyruvate in the 
blood pool can be used for normalization of myocardial metabolite signals (ie, bicarbonate). Reprinted from Lau et al75 with permission 
of the publisher. Copyright © 2010, John Wiley and Sons Inc.
of diabetic cardiomyopathy, although its diagnosis currently relies on 2 important components:95; the detection of myocardial abnormalities, usually including evidence of hypertrophy and left ventricular diastolic dysfunction determined by noninvasive echocardiographic or CMR methods, and the exclusion of other contributory causes of cardiomyopathy. The precise pathophysiological mechanisms leading to the development of diabetic cardiomyopathy also remain unclear.97 However, the altered myocardial substrate metabolism characteristic of diabetes mellitus has been linked with cardiomyopathy progression,96,98,99 suggesting that hyperpolarized 13C MR may play a role in clarifying the mechanisms of disease and in developing more specific diagnostic criteria.

In animal models of diabetes mellitus, the heart switches almost exclusively to fatty acids and ketone bodies for its ATP requirements, at the expense of glucose and pyruvate oxidation.23,99 This switch in substrate use is dominated by decreased PDH activity100–102 resulting from increased plasma free fatty acids. Hyperpolarized [1-13C]pyruvate with MRS has been used to measure in vivo cardiac substrate selection in rat models of acute streptozotocin-induced type 1 diabetes mellitus and insulin resistance induced by 28 days of feeding a high-fat diet. In type 1 diabetic rats, PDH flux was 65% lower compared with that in control rats and correlated with disease severity,32 whereas insulin-resistant rats had a 56% reduction in PDH flux.31 In the future, serial studies with hyperpolarized [1-13C]pyruvate, [2-13C]pyruvate, and potentially other metabolic tracers that target oxidative metabolism downstream of PDH should be performed to examine longitudinal changes of in vivo metabolism in chronic models of insulin resistance, metabolic syndrome, and diabetes mellitus. Collectively, these studies could more fully illustrate the metabolic alterations involved in the onset and progression of diabetic cardiomyopathy.

Hyperpolarized 13C MR measurements of cardiac glucose oxidative capacity (at the level of PDH flux) could optimize the use of novel therapies for diabetes mellitus and cardiomyopathy that improve glycemic control and normalize substrate selection such as glucagon-like peptide analogs103,104 and dipeptidyl peptidase 4 inhibitors.105 Additionally, clinical studies using cardiac hyperpolarized 13C MR methods to serially examine diabetic patients could reveal the role of altered substrate selection in compromising cardiac structure and function, recognize an early-stage metabolic profile indicating that a diabetic patient may be at risk for developing cardiomyopathy, and identify the profile of 13C substrate use, confirming that a patient’s heart failure is related to diabetes mellitus.

Other Metabolic Cardiomyopathies

Evidence is emerging that many patients with “idiopathic” cardiomyopathy have inherited defects in either mitochondrial energy metabolism or sarcomeric proteins.106 The so-called metabolic cardiomyopathies develop in the context of a wide spectrum of systemic pathological conditions. In many cases, patients present with symptoms of systemic disease, although in some conditions, including Anderson-Fabry disease (a disorder of lysosomal metabolism caused by α-galactosidase A deficiency), several types of glycogen storage disorders, inborn errors of fatty acid metabolism, and myopathic carnitine deficiency, patients may initially present with cardiomyopathy.1,106–108 Because of the rare occurrence of many metabolic cardiomyopathies and the poor correlation between in vitro enzyme assays and disease severity,106 initial diagnosis can be difficult and often can be made conclusively only by genetic evaluation.107

When hyperpolarized 13C MRI and MRS methods are approved for human use, determining the 13C metabolic profiles of patients with metabolic cardiomyopathies could help to clarify the specific biochemical defects that underlie the development of cardiac disease. Furthermore, in patients with systemic metabolic disorders that initially and predominantly manifest as a cardiomyopathy, 13C metabolic profiling could confirm the metabolic basis of disease and suggest the optimal course of treatment. As an example, in vivo metabolism in the hyperthyroid rat heart has been investigated with [1-13C]pyruvate and MRS.109 The term hyperthyroidism encompasses a heterogeneous group of disorders, all characterized by elevated levels of thyroid hormones and an increased basal metabolic rate. Hyperthyroidism causes many systemic effects that markedly affect the heart, increasing heart rate, contractility, and cardiac output; causing hypertrophy; and altering myocardial substrate selection.110 Hyperpolarized 13C MRS revealed a 55% reduction in PDH flux in hyperthyroid animals compared with controls, a result that helped to clarify one cause of thyroid hormone-induced cardiomyopathy.109

It is likely that carnitine deficiency may be detectable by following the conversion of hyperpolarized [2-13C]pyruvate into [1-13C]acetyl-carnitine, and simple carnitine supplementation reverses the symptoms of intractable congestive heart failure within 5 months.108 Additionally, glycogen storage disorders and Anderson-Fabry disease can be misdiagnosed as hypertrophic cardiomyopathy,1,107,111 and the different clinical courses associated with hypertrophic cardiomyopathy and metabolic cardiomyopathies underscore the importance of accurate diagnosis. For example, glycogen storage disease has been linked to progressive conduction system disease that may necessitate the implantation of a pacemaker and aggressive control of arrhythmias,107 whereas patients with Anderson-Fabry disease respond to enzyme replacement therapy.112 Metabolic phenotyping of these conditions with hyperpolarized 13C MRS could make an important clinical contribution to the diagnosis and management of such patients.

Future Directions and Challenges

Eventual translation of hyperpolarized 13C methods into the clinic will require considerable technological advances in terms of improved methods and hardware for the acquisition of 13C images. To identify focal regions of ischemia with lactate and/or pH measurements, for example, 3-dimensional images of [1-13C]lactate, H13CO3−, and 13CO2 with high spatial resolution will be required. Recently, a chemical shift–specific, cardiac- and respiratory-gated, multislice spiral MRI method was demonstrated with the capacity to map the distribution of [1-13C]pyruvate, [1-13C]lactate, and H13CO3− with 8.8-mm in-plane spatial resolution and 10-mm
slice thickness in normal pig hearts in vivo (voxel size, 0.774 mL), which suggests the feasibility of acquiring such data from patients (Figure 7). Furthermore, successful dynamic lactate imaging with a voxel size of 0.125 mL has been demonstrated in tumors, and despite the added challenges of acquiring MR images from the beating heart, it is likely that the spatial resolution attainable for dynamic 13C-lactate imaging will improve toward this level. Cardiac metabolic imaging will also benefit from the design and implementation of outer volume suppression radiofrequency pulses that ensure that metabolites generated outside the heart do not wash into the region of interest and confound studies of cardiac enzymatic fluxes.

Development of metabolic tracers beyond [1-13C]pyruvate, [2-13C]pyruvate, and 13C-bicarbonate will enable additional metabolic enzymes to be studied in the heart. Distinct spans of the Krebs cycle may be assessed in the heart with either hyperpolarized [1-13C]glutamate or [1,4-13C2]fumarate, the respective conversions of which to [1-13C]α-ketoglutarate and [1,4-13C2]malate have been demonstrated both in vitro and in vivo. Additionally, hyperpolarized [1,4-13C2]fumarate may emerge as a specific metabolic marker of cell death by necrosis. Hyperpolarized [1-13C]acetate may form the basis of an assay for intracellular CoA levels or, like the PET tracer 11C-acetate, may enable measurement of total metabolic capacity.

A recent proof-of-concept study has demonstrated that a “secondary hyperpolarization” approach, in which DNP hyperpolarization is transferred catalytically between molecules, may enable polarization of metabolites that otherwise would not polarize efficiently. This, along with other techniques using long-lived “singlet states,” may extend the hyperpolarized lifetime and therefore the potential imaging window. Furthermore, an increase in attainable polarization from the typical polarization levels of ∼30% described previously to ∼60% has recently been achieved by an increase in the magnetic field strength of the polarizing magnet and will aid the detection of low-concentration 13C-labeled metabolites and enable the development of new metabolic tracers.

Quantification of instantaneous metabolic fluxes will be essential for future development of both basic science and translational applications of hyperpolarized 13C MR. Basic science studies should strive both to measure metabolic fluxes in units that can be compared with conventional biochemical assays (ie, μmol·min⁻¹·g⁻¹) and to describe 13C flux through dynamic metabolic pools with rapid metabolic turnover where 13C may not accumulate such as [1-13C]citrate and [1-13C]acetyl-CoA. In the clinic, a system for monitoring the effects of the input function of the hyperpolarized agent on metabolic images must be developed because tracer pharmacokinetics could differ dramatically between control subjects and patients. A pulse sequence that dynamically images infused hyperpolarized [1-13C]pyruvate with a low flip angle has recently been demonstrated in pigs and is the first step toward input function quantification.

Finally, reproducibility studies to identify quantitative biomarkers that are both sensitive and specific to cardiovascular disease must be performed. Toward this goal, ratiometric markers that self-normalize to basal variations in tracer pharmacokinetics and metabolism may prove useful.

### Table. Potential Clinical Applications of Cardiac Hyperpolarized 13C Magnetic Resonance

<table>
<thead>
<tr>
<th>Application</th>
<th>Target</th>
<th>Proposed Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal heart</td>
<td>Optimize protocols</td>
<td>All tracers†</td>
</tr>
<tr>
<td></td>
<td>Understand normal metabolic fluxes</td>
<td>All tracers†</td>
</tr>
<tr>
<td></td>
<td>Demonstrate glucose–fatty acid cycle for drug targets</td>
<td>[1-13C]pyruvate</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>Understand acute ischemia/reperfusion injury</td>
<td>[1-13C]pyruvate and 13C-bicarbonate</td>
</tr>
<tr>
<td></td>
<td>Evaluate procedure</td>
<td>[1-13C]pyruvate and 13C-bicarbonate</td>
</tr>
<tr>
<td></td>
<td>Demonstrate coronary anatomy</td>
<td>Nonmetabolized tracers†</td>
</tr>
<tr>
<td></td>
<td>Demonstrate stress-inducible ischemia</td>
<td>[1-13C]pyruvate, nonmetabolized tracers†</td>
</tr>
<tr>
<td></td>
<td>Evaluate myocardial viability</td>
<td>[1-13C]pyruvate</td>
</tr>
<tr>
<td>Heart failure</td>
<td>Understand alterations in substrate metabolism</td>
<td>All tracers†</td>
</tr>
<tr>
<td></td>
<td>to aid patient management</td>
<td></td>
</tr>
<tr>
<td>Metabolic cardiomyopathies</td>
<td>Determine diabetic cardiomyopathy</td>
<td>[1-13C]pyruvate</td>
</tr>
<tr>
<td></td>
<td>Identify heritable metabolic cardiomyopathies</td>
<td>All tracers†</td>
</tr>
</tbody>
</table>

†Nonmetabolized tracers include 13C-urea and novel tracers optimized to remain within the vasculature and to have slow relaxation rates.

### Conclusions

When used with MRI and MRS, hyperpolarized 13C-labeled tracers offer the first method to measure cardiac substrate metabolism in real time and in vivo. The recent Food and Drug Administration approval given to hyperpolarized [1-13C]pyruvate for clinical studies of prostate cancer suggests that hyperpolarized 13C MR methods may be available for human studies of cardiovascular disease in the near future. Although the clinical applications of cardiac hyperpolarized 13C MR remain speculative, the technique has potential to advance basic knowledge, to improve diagnosis, and to optimize treatment of ubiquitous conditions such as myocardial ischemia and heart failure and rare diseases such as metabolic cardiomyopathies (Table). Future work with hyperpolarized 13C MR should focus on hardware and software development, data quantification, and development of new, highly polarized tracers to facilitate translation of the technology into the clinic and its application in clinical studies. It is possible that, in the future, hyperpolarized methods will form an important part of routine clinical assessment in cardiology.
Acknowledgments

For their constant hard work, enthusiasm, and support, we thank all the members of the Cardiac Metabolism Research Group, particularly Professor Sir George Radda, Dr Helen Atherton, Dr Lowri Cochlin, Daniel Ball, and Michael Dodd. We also thank Professor Lionel Opie, Dr Chuck Cunningham, and Dr Albert Chen for engaging in many helpful discussions.

Sources of Funding

We gratefully acknowledge the support of the British Heart Foundation (Program Grant RG/07/004/22659 to Prof Clarke, Program Grant RG/10/002/28187 to Prof Neubauer), the Wellcome Trust (Sir Henry Wellcome Postdoctoral Fellowship to Dr Schroeder), the Oxford BHF Centre for Research Excellence, and the Oxford NIHR Biomedical Research Centre.

Disclosures

Prof Clarke has received grant support from GE Healthcare. Dr Tyler has received grant support from GE Healthcare and Oxford Instruments Molecular Biotools. The other authors report no conflicts.

References


Hyperpolarized Magnetic Resonance: A Novel Technique for the In Vivo Assessment of Cardiovascular Disease
Marie A. Schroeder, Kieran Clarke, Stefan Neubauer and Damian J. Tyler

_Circulation_. 2011;124:1580-1594
doi: 10.1161/CIRCULATIONAHA.111.024919
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/124/14/1580

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/